Clinical studies of bone marrow failure

Sleijfer, Dirk Theodorus

CLINICAL STUDIES OF BONE MARROW FAILURE

D. TH. SLEIJFER

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STELLINGEN

I.

De nefrotoxiciteit van het cytostaticum cis-diammine-dichloro-platinum kan niet worden vastgesteld door bepaling van het serum-creatinine gehalte.

II.

Bij de diagnostiek naar afwijkingen van nieren en urinewegen bij pasgeborenen verdient echografie de voorkeur boven röntgenonderzoek.

III.

Indien bij een patiente een maligne ovariumtumor wordt vermoed, dient de buikinhoud te worden geïnspecteerd via een mediane onderbuiksincisie en niet via een Pfannenstiel-incisie.

IV.

De keuze van een bepaald antibioticum dient mede bepaald te worden door het effekt op de kolonisatie resistentie van de tractus digestivus.

V.

Cytostatische behandeling bij een gravida mag niet routinematig worden voorafgegaan door het afbreken van de zwangerschap.

VI.

D.M.S.O. (dimethyl sulfoxide) dient een plaats te krijgen in de behandeling van hersenoedeem.

VII.

Bij klinisch stadium I non-seminoma testistumor kan retroperitoneale klierdissectie achterwege blijven.

VIII.

Bij de lokale therapie van psoriasis dient de behandeling geen grotere frustratie op te leveren dan het ziektebeeld zelf.

IX.

Als bij een patient met schildkliercarcinoom de total body scintigrafie met ^{131}I negatief is geworden kan tijdens de controlefase volstaan worden met bepalingen van het serum thyreoglobuline gehalte.

X.

Het psychisch trauma dat kan ontstaan bij de behandeling met vloeibare stikstof van verrucae vulgares bij jonge kinderen wordt in het algemeen niet gerechtvaardigd door de ernst van de aandoening.

XI.

De resultaten van de adjuvans CMF-therapie na een mamma-amputatie wegens carcinoom, zijn niet afhankelijk van de menopauzale status van de patiente.

XII.

Bij verworven aplastische anemie kan een genetische predispositie van betekenis zijn.

XIII.

Nadere histochemische classificatie van amyloidosis kan op eenvoudige wijze geschieden door toepassing van de kaliumpermanganaat methode.

XIV.

De huidige neiging naar een afstammelingenverering in tegenstelling tot de vroegere ouderverering, hangt samen met de zorg en de angst voor de wereld die we onze kinderen nalaten.

Stellingen
behorende bij het proefschrift van
D. Th. Sleijfer

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Groningen 1981

RIJKSUNIVERSITEIT TE GRONINGEN

CLINICAL STUDIES OF BONE MARROW FAILURE

PROEFSCHRIFT

ter verkrijging van het doctoraat in de geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. M. R. van Gils
in het openbaar te verdedigen op woensdag 11 maart 1981
des namiddags te 3.00 uur precies
door

DIRK THEODORUS SLEIJFER

geboren te Leeuwarden

1981
DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotores:	Prof. Dr. H. O. Nieweg, F.R.C.P.
	Prof. Dr. D. van der Waaij
Co-promotor:	Prof. Dr. G. J. P. A. Anders
Referent:	Dr. N. H. Mulder

VOORWOORD

Dit proefschrift werd bewerkt in de afdeling Hematologie (hoofd: Prof. Dr. H. O. Nieweg) van de Kliniek voor Inwendige Ziekten (hoofd:Prof. Dr. E. Mandema) van het Academisch Ziekenhuis te Groningen, in samenwerking met de afdeling Bacteriologie (hoofd: Prof. Dr. D. van der Waaij) van het Laboratorium voor Medische Microbiologie (hoofd: Prof. Dr. J. B. Wilterdink) en het Anthropogenetisch Instituut (hoofd: Prof. Dr. G.J. P. A. Anders).

Hooggeleerde Nieweg, hooggeachte promotor, het is een voorrecht geweest te mogen werken aan een onderwerp dat Uw bijzondere belangstelling heeft. Bovendien is de periode die ik op Uw afdeling heb mogen doorbrengen van invloed geweest op mijn uiteindelijke beroepskeuze.

Hooggeleerde Van der Waaij, hooggeachte promotor, een belangrijk gedeelte van dit proefschrift is een uitvloeisel van Uw ideeën en van het vele werk dat hierover reeds door U werd verricht. Voor de hulp en de steun die ik van U mocht ontvangen ben ik U zeer erkentelijk.

Hooggeleerde Anders, hooggeachte co-promotor, de wijze waarop U steeds bereid was mij op de juiste ogenblikken terug te brengen op het moeilijke pad der genetica heb ik zeer op prijs gesteld.

Zeergeleerde Mulder, beste Nanno. De direkte aanleiding tot onderzoek naar diverse aspecten van het ziektebeeld aplastische anemie, was ons gezamenlijke verblijf in Londen, waar in de late avonduurtjes het idee ontstond dat „onze Groningse" resultaten ook maar eens geëvalueerd moesten worden. De blijmoedigheid waarmee je steeds over mijn spaarzame vrije tijd beschikte, sprak mij ergaan en voor jouw persoonlijke inzet bij het schrijven van alle „stukjes" ben ik je erg dankbaar. Ik prijs mij gelukkig, dat ook in de toekomst verdere samenwerking zal blijven bestaan.

Hilly de Vries-Hospers ben ik veel dank verschuldigd voor haar enthousiaste medewerking en inzet, zowel tijdens als na de diensturen.

Rik van Saene zorgde voor een opbeurend woord in moeilijke tijden en was een belangrijk man achter de schermen.

Zonder de steun van het verplegend personeel van de afdelingen Ao en Bo, het personeel van het hematologisch laboratorium en mevrouw T. Polling van de afdeling Klinische Bacteriologie - Streeklaboratorium voor de Volksgezondheid was dit proefschrift niet tot stand gekomen.

Mevrouw W. J. A. Bruins-van der Weij, lieve Willy, ik ben je erg dankbaar voor de snelheid en de nauwkeurigheid waarmee je alle versies de afgelopen tijd uit je typmachine toverde.

CONTENTS

INTRODUCTION

This thesis deals with some of the clinical aspects of the syndrome of bone marrow failure including aplastic anaemia. In the clinical entity of aplastic anaemia we have the classic prototype of bone marrow failure, but signs and symptoms of bone marrow failure which are the result of insufficient production of peripheral blood cells - red cells, granulocytes, monocytes and platelets - may occur in other disorders involving the bone marrow.

Aplastic anaemia as a separate clinical entity was first described by Ehrlich in 1888 (1). Absence of universally accepted criteria for the diagnosis of aplastic anaemia has contributed to confusion in classification, analysis of aetiology and interpretation of therapeutic results. At present a decrease in the cellularity of the marrow is considered essential for the diagnosis: sometimes the occurrence of bone marrow hypocellularity is only based on aspiration smears (2), but nowadays it is accepted that one (3, 4, 5, 6) or two or more (7) bone marrow biopsy specimens should exhibit moderate to severe aplasia to conform the diagnosis of aplastic anaemia. The definition of pancytopenia varies also widely: haemoglobin level lower than 9 g/100 ml (8) or lower than 13.5 g/100 ml (9), granulocyte counts below 1.5×10^9/l (8, 10) or below 2.5×10^9/l (9, 11), platelet counts below 75×10^9/l (8) or below 150×10^9/l (9). Most reports do not use the term aplastic anaemia in patients with bone marrow damage secundary to radiation or to chemotherapeutic agents (3, 4, 6, 8, 10, 11), or in patients with evidence of marrow-infiltrative processes or myelofibrosis (4, 6, 8, 10) and in patients with any blast cell excess in the bone marrow (8, 10, 12).

It is thought that in aplastic anaemia there may be a wide variety of pathogenic mechanisms which give rise to a disturbance within the haematopoietic system. Sometimes an inherent predisposition to bone marrow failure is present. In Fanconi's anaemia progressive bone marrow failure is combined with a complex pattern of congenital malformations. This syndrome has an autosomal recessive inheritance pattern. The X-linked recessive syndrome of dyskeratosis congenita frequently includes the development of pancytopenia, while anaemia and reticulocytopenia are a part of the autosomal recessive Diamond-Blackfan anaemia (6). These examples show that the normal haematopoiesis is to a far extent a genetically controlled process failing in all the above described cases at very specific points. It seems even possible that in some patients with an acquired aplastic anaemia the aplasia results from the combination of a toxic insult and a

genetically conditioned sensitivity of the bone marrow. In this connection the observations about the familial occurrence of acquired aplastic anaemia described in chapter I are of interest.

In nearly one half of the cases of acquired aplastic anaemia the disease is related to drugs and other chemicals (6, 13). Some of these agents, such as benzene and phenylbutazone can produce chromosomal damage in the stem cells. This may lead eventually to acute leukaemia (14). In one of our patients with aplastic anaemia leukaemia developed after the bone marrow had seemingly recovered from the aplasia thought to be induced by massive use of analgesics (chapter II). The majority of the remaining cases of acquired aplastic anaemia is called idiopathic, which means that no specific aetiological agent can be found. Rarely there is an association with thymoma (15), infection, particularly viral hepatitis (16, 17) or pregnancy (1, 18).

Although some of the exogenous factors leading to aplastic anaemia have thus been determined, the actual mechanism which causes the bone marrow insult is as yet not always clear. In general bone marrow aplasia may be said to be the result of defective stem cells or defects in the micro environment. Insight into the function and development of the stem cells has been gained by the use of in vitro methods (19). By means of the soft agar colony assay for granulocyte-monocyte progenitors (CFU-c) it was demonstrated that some patients with aplastic anaemia appear to have grossly reduced numbers of granulocyte precursors in the bone marrow, suggesting stem cell defects (20, 21). Successful repopulation of the patients marrow with normal donor stem cells in a number of cases (22, 23) also indicates a deficiency or defect in the patient's own stem cells. Some reports mention the value of in vitro technics to study drug-induced pancytopenia (24, 25), while others do not think that these technics will be useful in diagnosing drug-induced aplasia (26). Destruction of the stem cell micro environment may also lead to aplastic anaemia, as was suggested by Knospe e.a. (27). A defect in the micro environment is also possible in those patients with aplastic anaemia with a normal in vitro myelopoiesis (21). On the other hand, ultrastructural studies of the bone marrow sinusoids in patients with aplastic anaemia failed to provide evidence for this hypothesis (28).

An increase of lymphocytes and plasma cells in marrow specimens of patients with aplastic anaemia (29) as well as reports about the beneficial results of treatment with antilymphocyte globulin (30), prednisone (31) or cyclophosphamide (32) in aplastic anaemia suggest an auto-immune process. Suppression of colony formation of normal marrow cells by marrow

from patients with aplastic anaemia also seems to support the hypothesis of an immune-mediated disease (21).

In view of the many different aetiological factors and their variety of effects on the marrow, it is not surprising that aplastic anaemia has a highly variable clinical course. The median survival ranges from 6 (33) to 24 months (4). Some patients die within a few weeks, others survive for years with persistent pancytopenia, and ten per cent of all patients show a complete recovery (3). The fact that there are now different therapeutic modalities (5, 7, 22, 30, 31, 32, 34) has resulted in the problem of selecting the appropriate treatment for patients. In order to determine the eventual course and outcome of aplastic anaemia increasing attention has been paid to single prognostic factors: age, sex, symptoms, granulocytopenia, thrombocytopenia, reticulocytopenia (4, 8, 10, 12, 17, 29) or to a combination of these factors (11, 12, 35). We were especially interested in this problem of patient selection because aggressive methods of treatment have been advocated in recent years (chapter III).

The recent improvements in the treatment of patients with bone marrow failure, whether induced by cytostatic treatment or resulting from aplastic anaemia, or infiltration of the bone marrow by fibrosis or malignancy, have stimulated interest in the diagnosis, management and prevention of disease- or treatment-related complications. Bleeding and infection are the main life-threatening complications. Bleeding is most often the result of thrombocytopenia, and the risk of serious bleeding increases if platelets fall below $5 \times 10^9/l$ (36). Platelets can be provided on a prophylactic basis, but the advantages of this approach must be balanced against a more rapid development of antibodies, resulting in a diminished benefit from these transfusions in possible emergencies. Granulocytopenia is perhaps the most important factor which enhances the susceptibility of these patients to infections. Bodey et al (37) have documented the relationship between the degree and duration of granulocytopenia with the incidence of infections. While the incidence of infections depends on the degree of granulocytopenia the development of infections in patients with bone marrow failure can also promote a haematologic relapse (38). Infection remains the most common cause of death in patients with acute leukaemia (39) or with aplastic anaemia (3), despite the use of combinations of two or more types of bactericidal antibiotics. The majority of the bacterial infections in these patients are caused by endogenous gram-negative bacteria (40) and various techniques have been explored to prevent these infections. Reverse isolation to

reduce acquisition of new micro-organisms may be of some value in preventing infections; reverse isolation in combination with prophylactic oral non-absorbable antibiotics, however, appears to reduce dramatically the incidence of infections (41). The oral use of non-absorbable antibiotics alone has led to conflicting data with regard to infection prevention (41). As a result of the destruction of the colonization resistance (42), the likelihood of acquisition of resistant micro-organisms leading to colonization and fatal infections is great. We have studied the value of orally administered antibiotics that suppress growth of potentially pathogenic micro-organisms, but leave the colonization resistance of the alimentary tract unaffected as a method of infection prevention in patients with granulocytopenia due to aplastic anaemia or due to cytostatic therapy. The results of this randomized study are reported in chapter IV.

Although the clinical picture of the bone marrow insufficiency as a result of aggressive treatment in haematology and medical oncology is nowadays quite common, aplastic anaemia in the restricted sense is a relatively rare disorder. Our knowledge of supportive measures for patients with bone marrow failure is increasing and this will result in a prolonged survival for most of these patients. However, these measures do not have a therapeutic effect on the underlying disease, and patients with aplastic anaemia are still treated with a variety of therapeutic modalities in consequence of the lack of clear understanding of the pathophysiological mechanisms. Probably carefully designed laboratory investigations and controlled clinical studies will increase our knowledge of the pathophysiology and the therapy of aplastic anaemia.

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Acta Med Scand 207: 397-402, 1980

CHAPTER I

ACQUIRED PANCYTOPENIA IN RELATIVES OF PATIENTS WITH APLASTIC ANAEMIA

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ABSTRACT

We studied relatives of adult patients with acquired aplastic anaemia. Eight patients were found to have 11 family members with peripheral blood pancytopenia. Six of the 11 affected relatives had diminished and four normal cellularity and one had hypercellularity of the bone marrow. Thus, 19 persons in these eight families were affected. In two families, father and son were affected, in four families brother and/or sister, in one family a brother and an aunt and in one a nephew of the index patient. None of the patients or family members had congenital defects. All patients were diagnosed at an adult age and, furthermore, also the mode of inheritance in some of the families seems to exclude Fanconi's syndrome. It is concluded that relatives of patients with aplastic anaemia should be screened for manifestations of this syndrome of familial acquired blood pancytopenias.

INTRODUCTION

Aplastic anaemia in childhood can be familial (4, 5) or isolated. Most cases seem to be constitutional, but some may be acquired. In adults, however, aplastic anaemia is usually considered to be isolated and acquired, either drug-induced, postviral or idiopathic (15).

In a retrospective study of 55 adult patients with aplastic anaemia, we noticed a familial occurrence of pancytopenia in eight. This report describes these eight affected families.

PATIENTS AND METHODS

As many family members as possible of the eight patients with aplastic anaemia giving a positive family history of blood diseases were investigated.

Routine peripheral blood counts were performed by standard laboratory methods. Reticulocyte counts were corrected for haematocrit, 48% in men and 41% in women. Pancytopenia was defined as a haemoglobin level below 12.0 g/100 ml, a number of polymorphonuclear neutrophils below 2.500/mm³ and of platelets below 120.000/mm³ at the same time.

Bone marrow specimens were obtained by aspiration and by Yamshidi biopsy needle. Aplastic anaemia was diagnosed when a patient with pancytopenia turned out to have a hypocellular or an acellular bone marrow.

At physical examination, special attention was paid to the known stigmata of Fanconi's syndrome: short stature, skin pigmentation, microcephaly, ocular anomalies, cardiovascular anomalies and hypogonadism. Skeletal malformations were investigated by physical examination and in most patients by X-rays of the forearms and hands. Information concerning the kidneys was obtained by i.v. pyelography.

Additional investigations included Ham test and liver function tests. In some patients cytogenetic studies were carried out with G-banding.

RESULTS

Anaemia and leukopenia and/or thrombocytopenia were found in 11 out of 26 relatives of the eight index patients with aplastic anaemia. Pertinent data on the index patients and their relatives with blood disorders are given in Table I.

Table I. *Data concerning index patients (A) and their relatives (B and C) with familial pancytopenia*

Pat. no.	Age (y.)	Sex	Aetiology	Hb (g/100 ml)	Reticulocytes[a] (%)	Leukocytes (per mm³)	Neutrophils (%)	Platelets (per mm³)	Bone marrow
1 A	30	♂	Drug-induced	10.8	0.68	2 500	85	32 000	Acellular
1 B	27	♀	Drug-induced	7.2	2.10	700	49	10 000	Hypocellular
1 C	19	♀	Drug-induced	11.8	1.56	1 500	34	70 000	Acellular
2 A	81	♀	Drug-induced	7.4	0.60	1 000	17	7 000	Acellular
2 B	67	♂	Drug-induced	5.2	1.00	1 500	35	37 000	Hypocellular
3 A	36	♂	Idiopathic	9.7	1.32	3 000	50	25 000	Hypocellular
3 B	63	♂	Idiopathic	6.4	–	1 000	12	10 000	Acellular
4 A	42	♂	Drug-induced	7.7	1.00	2 400	60	3 000	Acellular
4 B	29	♂	Drug-induced	10.7	0.96	3 200	32	14 000	Normocellular
5 A	75	♂	Idiopathic	11.4	0.87	2 600	21	58 000	Acellular
5 B	71	♂	Drug-induced	6.6	1.22	1 200	35	667 000	Hypercellular
5 C	72	♂	Idiopathic	5.7	2.08	4 000	56	42 000	Normocellular
6 A	27	♂	Idiopathic	4.0	0.71	700	31	3 000	Hypocellular
6 B	23	♂	Idiopathic	9.4	0.17	2 400	35	98 000	Hypocellular
7 A	17	♂	Drug-induced	7.6	0.27	1 500	9	5 000	Acellular
7 B	37	♀	Idiopathic	7.4	0.56	1 500	52	10 000	Normocellular
7 C	17	♂	Idiopathic	7.7	0.33	650	22	3 500	Acellular
8 A	21	♂	Idiopathic	6.5	0.16	2 900	51	79 000	Hypocellular
8 B	50	♂	Idiopathic	10.8	1.25	3 500	53	110 000	Normocellular

[a] Corrected for haematocrit, 48% in men and 41% in women.

All index patients had pancytopenia and hypoplasia or aplasia of the bone marrow without evidence of malignancy, extensive fibrosis or storage disease. Pancytopenia was present in ten relatives, bicytopenia in one. Hypoplasia or aplasia of the marrow was found in six relatives, normal cellularity in four and hypercellularity in one.

Physical examination did not reveal Fanconi stigmata in any of the index patients or relatives. In four patients no information concerning the kidneys could be obtained. In all others, i.v. pyelography was performed or the kidneys were found to be normal at autopsy. X-rays of the forearms and hands were available in nine patients and were all normal. None of the patients had hepatomegaly, splenomegaly or multiple lymphadenopathy.

In the majority of patients, paroxysmal nocturnal haemoglobinuria was excluded by the history, by the Ham and sucrose haemolysis tests. None of the patients showed evidence of thalassemia or recent hepatitis.

Cytogenetic studies, performed in three patients, disclosed a Philadelphia chromosome during the terminal stage of the disease in one of them.

Nine patients admitted use of potential aetiological agents. They included tolbutamide, sulfadimethoxine, diphenylhydantoin, indomethacin, salicylates, hexachlorocyclohexane and other insecticides, nitrofurantoin, trimethoprim-sulfamethoxazole and Dolviran®. In one of these nine patients, 7A, we found serological evidence of a recent influenza A virus infection. No aetiological factor could be found in the others.

CASE HISTORIES

Family 1

Patient 1A was seen for the first time in 1967 at the age of 27 years because of diabetes mellitus. He was treated with diet and tolbutamide. Peripheral blood cell counts were normal until 30 years of age, when pancytopenia was discovered. Bone marrow biopsy revealed an acellular marrow. Tolbutamide treatment was discontinued. During the following 8 years the aplastic anaemia was unchanged. A recent bone marrow biopsy again showed an acellular marrow.

Patient 1B, a sister of 1A, had been treated repeatedly with sulfadimethoxine for urinary tract infections. In 1961 she exhibited pancytopenia. Her bone marrow was hypocellular. Therapy with vitamin B_{12}, folic acid and other vitamins, corticosteroids and anabolic steroids was unsuccessful. In the following years the patient experienced urinary and respiratory tract infections and allergic reactions to penicillin and iodopanoic acid. She died in

1967 of Escherichia coli septicaemia. A hypocellular bone marrow was found at autopsy.

Patient 1C, a sister of 1A, received phenobarbital, diphenylhydantoin and caffeine for epilepsy diagnosed at the age of 14 years. In 1964, at the age of 19, she developed spontaneous haemorrhages and was found to have a pancytopenia. Bone marrow biopsy revealed an acellular marrow. No treatment was instituted, except discontinuation of diphenylhydantoin. The pancytopenia still persists.

Physical examination and peripheral blood cell counts in two brothers did not show any abnormalities.

Family 2

Patient 2A was seen in 1975, at the age of 81 years, when she complained of fatigue. In the years prior to admission, she had used indomethacin and salicylates for degenerative arthritis. Bone marrow biopsy disclosed an aplastic marrow. Prednisone therapy was unsuccessful. She died recently and the cause of death was not established by autopsy.

Patient 2B, a brother of 2A, was first admitted in 1965 at the age of 67 years. He suffered from degenerative arthritis and used abundant analgesics, especially acetylsalicylic acid. Pancytopenia was diagnosed, his bone marrow was hypoplastic. He was treated with anabolic steroids and blood transfusions, but the pancytopenia deteriorated gradually. His bone marrow was repeatedly found to be hypoplastic. The patient died in 1973.

Results from physical examination and laboratory studies in four sisters and one brother were normal. One sister and two brothers could not be examined.

Family 3

Patient 3A was admitted for the first time in 1971 with a duodenal ulcer. Thrombocytopenia (55.000/mm^3) was found by chance. A marrow aspiration was normal. In 1972, at the age of 36 years, he had developed pancytopenia and the bone marrow was hypocellular. In 1977, a bone marrow biopsy showed an acellular marrow. Results of cytogenetic studies of peripheral blood were normal.

Patient 3B, the father of patient 3A, was seen at the age of 61 because of bleeding after a dental extraction. He was anaemic without leukopenia or thrombocytopenia, but two years later, in 1967, pancytopenia was present. Bone marrow biopsy showed an acellular marrow. During hospitalization he

received blood transfusions, and corticosteroids, but he died of myocardial infarction after a few weeks.

Patient 3A has no siblings. Information concerning brothers and sisters of patient 3B is lacking.

Family 4

Patient 4A was admitted for the first time in 1973 at the age of 42 years because of pancytopenia and a bleeding diathesis. As a farmer he had used the insecticide hexachlorocyclohexane in the months prior to hospitalization. Bone marrow biopsy was acellular. Treatment with anabolic steroids was unsuccessful: the pancytopenia gradually deteriorated. Six months later the patient died of Escherichia coli septicaemia and cerebral haemorrhage.

Patient 4B, a son of a sister of patient 4A, was seen in our hospital in 1975 at the age of 29 years because of pancytopenia. Until this time he had been well. As a farmer he also had frequently used insecticides. Bone marrow biopsies until now show a normal cellularity. The patient has not been treated, and blood cell counts remain at the same level.

Information concerning the other family members could not be obtained.

Family 5

Patient 5A was first admitted in 1960 at the age of 62 years, when the diagnosis of pernicious anaemia without leukopenia and thrombocytopenia was made. Treatment with vitamin B_{12} injections was started followed by a rapid response. At 75 years of age pancytopenia was discovered. The patient did not use drugs other than vitamin B_{12} injections. The bone marrow was acellular without megaloblastic haematopoiesis. There was no response to treatment with vitamin B_{12} or folic acid. The pancytopenia deteriorated gradually and the patient died two years after the diagnosis of aplastic anaemia. The cause of death was not determined by autopsy.

Patient 5B, a brother of patient A, was seen in 1976 at the age of 71 because of fatigue and weakness. He had been treated with nitrofurantoin and a combination of trimethoprim-sulfamethoxazole for urinary tract infections. The platelet count was normal but he was anaemic and had leukopenia. A bone marrow aspiration and a biopsy showed a hypercellular marrow. Cytogenetic bone marrow studies showed normal results. Repeated bone marrow investigations in 1977 again showed hypercellularity in both erythropoiesis and myelopoiesis, and also megakaryocytosis. The anaemia and leukopenia persisted in the peripheral blood. The patient died recently of pulmonary embolism.

Patient 5C, a brother of patient 5A, was admitted to our hospital in 1968 at the age of 72 because of weakness and dyspnoea. Pancytopenia was discovered. Bone marrow aspiration and biopsy showed normal cellularity without megaloblastic haematopoiesis. Treatment with vitamin B_{12}, folic acid and pyridoxin was not effective. The blood pancytopenia deteriorated and the patient died in 1970 of gastric haemorrhage.

Another brother has normal peripheral blood cell counts. Two sisters died in 1918 at the age of 16 and 22 years of an influenza infection.

Family 6

This is the only family with a history of consanguinity. The parents of the index patient were first cousins.

Patient 6A was seen in 1972 at the age of 25 years because of bleeding after tooth extraction. He had leukopenia and thrombocytopenia, but still a normal haemoglobin level. The bone marrow biopsy showed normal cellularity. In 1974 the pancytopenia deteriorated: haemoglobin 4.0 g/100 ml, WBC 700/mm^3, platelets 3.000/mm^3. The patient died in 1975 of Gram-negative bacterial septicaemia. At autopsy the bone marrow was found to be severely hypocellular.

Patient 6B, a brother of 6A, was first admitted in 1971 at the age of 23 because of pallor and fatigue. He had pancytopenia that deteriorated gradually. The bone marrow was hypocellular. Administration of vitamin B_{12}, folic acid, pyridoxin and anabolic steroids was unsuccessful. Three years later the bone marrow was again hypocellular. In 1975, blast cells appeared in the peripheral blood. The patient developed high fever and died. Premortal cytogenetic studies of peripheral blood disclosed a Philadelphia chromosome in one of six analyzed metaphases. Autopsy revealed a hypercellular bone marrow with predominantly large, abnormal blast cells, while pulmonary haemorrhages and fungal abscesses in the lungs and the brain were considered to be the cause of death.

One brother had normal peripheral blood cell counts and a normal marrow on biopsy. A sister had normal haemoglobin level and normal number of platelets, but granulocytopenia, 42% of 4.000 white cells/mm^3. A bone marrow aspiration revealed normal cellularity.

Family 7

Patient 7A had been well untill 1977 when he was hospitalized for the first time at the age of 17 because of pancytopenia with fever and a bleeding

diathesis. In the week prior to admission he had used phenethicillin and Dolviran® (salicylate, phenacetin, codeine, caffeine and phenobarbital) for an influenza A virus infection. Marrow aspiration and bone marrow biopsy showed a nearly acellular marrow. Four weeks after admission the patient was transferred to the University Hospital, Leiden, for bone marrow transplantation. He died of a disseminated fungal infection before bone marrow transplantation could be performed. Autopsy revealed an acellular marrow.

Patient 7B, a paternal aunt of patient 7A, was seen in 1976 at the age of 37 in the University Hospital, Utrecht (K. Punt) because of pancytopenia and hypermenorrhoea. The bone marrow was reported to show a normal erythropoiesis and myelopoiesis but no megakaryocytes. Treatment with corticosteroids and anabolic steroids was unsuccessful.

Patient 7C was investigated in 1977 during the hospitalization of his brother, patient 7A. At that time his peripheral blood cell counts were normal: haemoglobin 12.8 g/100 ml, leukocytes 4.000/mm^3 with a normal differential count, and platelets 203.000/mm^3. In 1978, at the age of 17, he was admitted to the University Hospital of Leiden (J. M. Vossen) because of a bleeding diathesis, and pancytopenia was diagnosed. Bone marrow biopsy revealed an acellular marrow. The patient died two months after bone marrow transplantation as a result of graft versus host disease.

The father of patient 7A had normal haemoglobin level and platelet count, WBC was 4.000/mm^3 with 45% neutrophils.

One sister of patient 7A who was HLA-identical, MLC-negative, also had a normal haemoglobin level and platelet count. Her WBC was 5.600/mm^3 with 33% neutrophils. The mother and two sisters had normal peripheral blood cell counts. Patient 7B has six sisters and five brothers (the father of patient 7A included). They have no clinical complaints. Laboratory studies are, however, not available.

Family 8

Patient 8A was seen in our hospital in 1970 because of pancytopenia. The bone marrow was hypocellular. He was treated with anabolic steroids without any effect on haemoglobin level or the number of leukocytes. Platelet count, however, was normalized.

Patient 8B, father of patient 8A, was hospitalized because of pancytopenia in 1970 at the age of 50. He had a history of anaemia since 1955. Marrow aspiration and biopsy showed normal cellularity. Two years later the patient died of a dissecting aneurysm. The bone marrow was normal at autopsy.

Patient 8A has one sister who has no complaints. Information is lacking on family members of patient 8B.

DISCUSSION

Constitutional aplastic anaemia can be familial as described by Fanconi (5) in 1927. In all of these cases the blood disorder is associated with multiple congenital malformations. Estren and Dameshek (4) described in 1947 a second group of children with familial aplasia but without congenital defects. Chromosomal anomalies have been described subsequently in both groups (11, 16).

Acquired aplastic anaemia has rarely been recorded in more than one member of a family. Four pairs of twins with chloramphenicol-induced aplastic anaemia have been reported (9, 10). Two siblings became pancytopenic after infectious hepatitis (2). In another family, two sisters developed aplastic anaemia after administration of gold compounds (3). McLaren et al. (8) reported two sisters who developed transient aplastic anaemia induced by methyprylon. Three cases of idiopathic acquired aplasia in one family were described by Stolte (13) and one case of an affected mother and child is mentioned by Keiser (7). Though some of these patients were quite young, it seems acceptable that the pancytopenia was acquired in all of them and not constitutional.

Our patients also appear to have acquired and not constitutional disease. The stigmata of Fanconi's syndrome were always absent. In families 1, 2 and 4 the history indicated exposure to a drug or chemical known to induce aplastic anaemia. Other arguments for an acquired pancytopenia can be found in most other families: the patients in families 3 and 5 presented initially without complete pancytopenia, and members of families 6 and 7 passed the childhood without any sign of blood disease. Furthermore, one of the affected members of family 8 had not developed pancytopenia at 35 years of age. We must therefore assume that all of our patients have acquired aplasia, which is a rather rare disease throughout the world with an estimated incidence of about 4.8/1.000.000 persons/year (14). In our region the estimated frequency is approximately 6/1.000.000 persons/year, implying that its occurrence in more than one family member and in so many families can hardly be coincidental. It indicates that the members of the families described in this study probably have a genetically determined predisposition that makes them susceptible to development of bone marrow disease. In nine of our patients the expression of this predisposition seems to be

triggered by drugs. No clear-cut toxic agents have been found in the others. It might be suggested that these patients may react with bone marrow depression to agents not frequently associated with aplastic anaemia, for instance salicylates and influenza infection.

The expression of this predisposition to bone marrow disease in these families is most often in the form of depression: blood pancytopenia was present in all patients. However, a hypo- or aplasia of bone marrow elements was not found in all patients. This may be due to a variability of marrow cellularity that is often found in aplastic anaemia (6). In patients 3A and 6A, for example, we initially found a normal bone marrow cellularity, but later on a hypocellular and ultimately an acellular marrow. It is therefore possible to observe decreasing cellularity when the disease deteriorates. On the other hand, the absence of hypocellularity in some relatives probably suggests a disorder different from the usual aplastic anaemia. This is also suggested by the rather favourable prognosis of the patients and their relatives as far as median survival is concerned. That the expression of the genetic predisposition may not always lead to depression of the bone marrow is suggested by the course of the disease in patients 6B and 5B. In the former patient, terminal evidence is found for the proliferation of blast cells, in the latter patient, proliferation of all cell lines of the bone marrow was present resulting in marked thrombocytosis. This option of the bone marrow to react either with aplasia or proliferation is also known in benzene intoxication and in patients with the constitutional childhood aplasia of Fanconi, who rather frequently develop some form of leukaemia (1).

The incidence of familial pancytopenia in our region seems to be remarkably high. In the period during which the patients were seen, 47 other patients without family history were admitted to our hospital. This would mean that 15-20% of our patients with aplastic anaemia have family members with blood dyscrasias. It is clear that this percentage is strikingly different from what is known in the literature. Moreover, in the future some of the isolated cases may probably turn out to be familial.

The selection of our study population was of course biased by the fact that our attention was drawn to the families by at least one patient with clinical symptoms of pancytopenia and that only those families in which more than one patient was found are described in this report. Nevertheless, we are confronted with an unusual and striking familial occurrence of pancytopenia in adults. Possibly a genetic defect could be the origin of these abnormalities. Pancytopenia in children, Fanconi's anaemia, has a well defined genetic

origin as an autosomal recessive gene mutation (12). Recessive heredity practically always implies a horizontal clustering of disease cases in families. Among the eight families reported here there were four with an additional vertical expansion, two families with father and son, one family with an affected aunt of the index case and another with a nephew of the index case who had pancytopenia. But when the methodical bias in the gathering of our material is taken into account, there are too few patients to allow for a hypothesis of plain autosomal dominant inheritance.

Considering that the age at onset varies widely and that a large proportion of our patients manifested the disease only after having been exposed to haematoxic agents, it seems not unreasonable to consider the possibility of a dominant susceptibility mutant with variable penetrance, or of a multifactorial inheritance with threshold. The latter possibility is much more hypothetical than the former because any reliable data about population frequency or suitable amount of family data are lacking up to now.

After all, it seems reasonable to suppose that if there is a genetic background to these cases of pancytopenia, it is different from that of Fanconi's anaemia. Consistent with this difference is the absence of the chromosomal fragility described in Fanconi's anaemia in the cases we could investigate.

If, in the future, a genetic defect proves to be of any importance for the development of some cases of adult pancytopenia, both the fact that the age distribution of our patients at the onset of the disease seems to be discontinuous and the marked similarity in the age at onset within the individual families would be interesting.

For practical purposes it seems to be important to question patients with aplastic anaemia about their relatives and to examine these relatives for blood dyscrasias, especially if they are considered as possible bone marrow transplantation donors.

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CHAPTER II

ACUTE LEUKEMIA AFTER ACQUIRED APLASTIC ANEMIA

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ABSTRACT

The case histories of two patients with acquired aplastic anemia are reported. After treatment with oxymethalone and prednison with methandrostenolon respectively, a hematologic remission was achieved in both patients. However, after a remission period of several years duration both patients developed an acute myeloid leukemia. The implications of the relation between aplastic anemia and acute leukemia are discussed.

INTRODUCTION

Acquired, non constitutional aplastic anemia has only rarely been reported to terminate in acute leukemia. Most of these reports concern cases of leukemia developing during the course of benzene-induced aplastic anemia (1, 5, 16).

We report two patients with acquired aplastic anemia, one idiopathic, the other probably induced by acetylsalicylic acid. After a sustained remission the patients developed acute myeloid leukemia six and eleven years respectively after aplasia was first diagnosed.

CASE REPORT

Patient I was first hospitalized in 1970 at the age of 13 because of anemia and a bleeding diathesis. His past history was unremarkable, there was no history of exposure to agents related to bone marrow depression. On physical examination no stigmata of Fanconi's anemia were found. Liver, spleen and lymphnodes were not enlarged. Intravenous urography showed a normal urinary tract, X-rays of the hands and fore-arms were normal. Laboratory data included a hemoglobin level of 5.5 g/100 ml, with 11 per cent fetal hemoglobin, a reticulocyte count of 0.2%, white blood cell count of $2{,}9 \times 10^9$/l with 14% neutrophils and 78% lymphocytes, the platelet count was 4×10^9/l. Bone marrow biopsy showed a severely hypocellular marrow

on two different occasions. A diagnosis of idiopathic, acquired aplastic anemia was made and the patient was treated with oxymetholone in dosages varying between 20 and 60 mg daily. During this treatment his hemoglobin level rose to 7.1 g/100 ml, the white blood cell count was stable but the platelets had risen to 90×10^9/l. At the age of 19 he was hospitalized again. His growth pattern had been normal. Although the patient had no specific complaints, the character of his disease seemed to have changed. The differential count of 1.5×10^9 white blood cells/l showed 15 per cent immature blast cells. A few weeks later the blast count had risen to 35 per cent. A bone marrow biopsy showed a hypercellular marrow with a disturbed myelopoiesis, however leukemia was not evident. After half a year of observation without therapy, the hemoglobin level had dropped to 4.0 g/100 ml, platelets to $17{,}5 \times 10^9$/l, while the white blood cells ($1{,}5 \times 10^9$/l) showed 45% blast cells. Cytochemistry is shown in table I. The bone marrow was found to con-

Table I. Cytochemistry of leukemic peripheral blood and bone marrow cells.

	Cytology	Sudan Black B	Peroxidase	Periodic Acid-Schiff	Esterase	Esterase, inhibited by fluoride
Peripheral blood leukocytes patient I 12-5-1976	Monomyeloblastic	+	±	—	+	—
Peripheral blood leukocytes patient II 21-2-1975	Monomyeloblastic	±	±	—	++	±
Bone marrow patient II 28-2-1975	Monomyeloblastic	±	±	—	++	±

tain 90% immature blast cells. The patient was treated with vincristin and prednison without a consistent effect. Despite the lack of result of the treatment, the disease had a somewhat protracted course, the patient died six months after the diagnosis of leukemia in the bone marrow had been established. Autopsy was not performed.

Patient II was referred to our hospital in 1963 at the age of 25. In the month preceding admission she had experienced a gradually increasing fatigue. Her past history showed an extensive use of acetylsalicylic acid (between 250-500 g in a ten year period), but was otherwise unremarkable. No other drugs were used. Physical examination was normal except for pallor. There were no stigmata of Fanconi's anemia. Liver, spleen, and lymph nodes were not enlarged. Initial laboratory data included a hemoglobin level of 7.4 g/100 ml, a reticulocyte count of 2%, white blood cell count of $3{,}9 \times 10^9$/l with 44% neutrophils and 52% lymphocytes. The

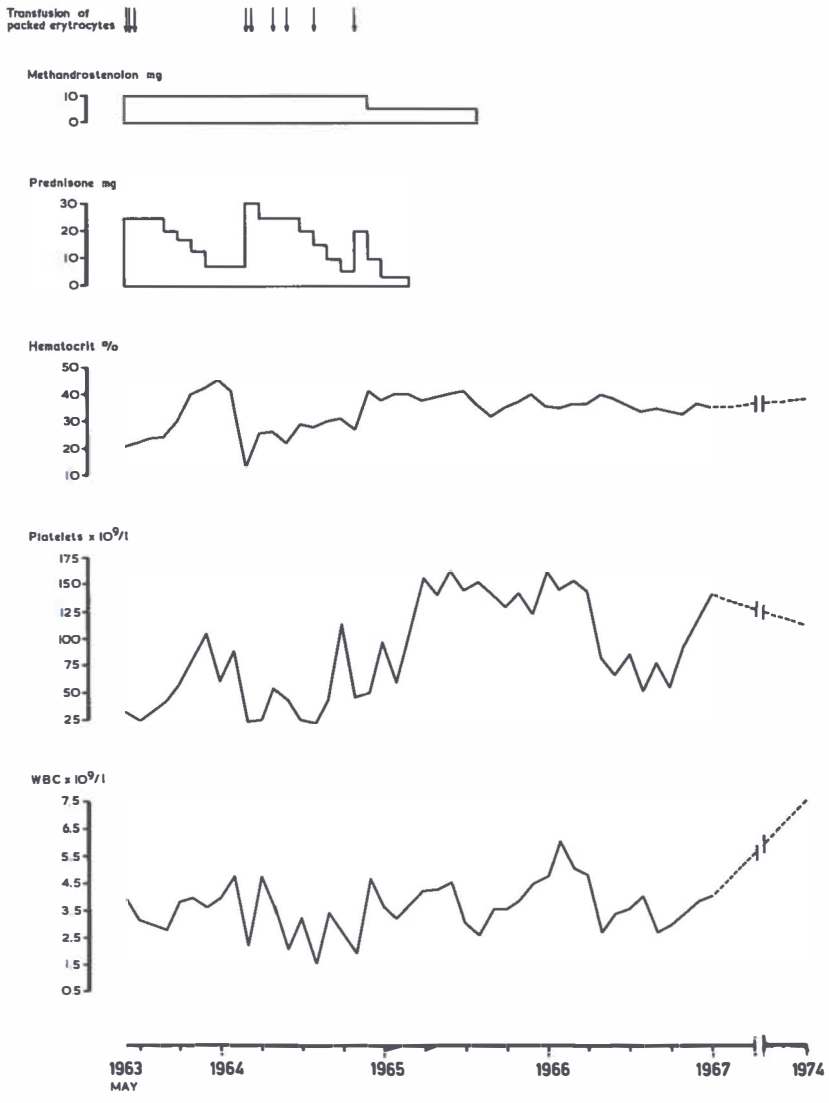

Figure 1. Therapy from 1963 till 1974, and course in hematocrit, platelets and WBC in the same period.
No substantial changes in blood parameters between 1967 and 1974 were recorded.

platelet count was 32,5 × 10^9/l. The life span of radiochromate labelled platelets was normal, seven days. Fetal hemoglobin was 0%. Paroxysmal nocturnal hemoglobinuria was excluded by a negative Ham-test. Liver function tests were normal, tests for autoimmunity were all negative. The bone marrow aspirate and biopsy was hypocellular with a predominance of mature lymphocytes. A diagnosis of acquired aplastic anemia was made. During hospitalization the patient was treated with blood transfusions and daily oral doses of prednisone and androgens. The course in variations in blood cell counts and therapy till 1967 is outlined in figure 1. Complications from the prednisone therapy included signs of Cushing's disease, an impaired carbohydrate tolerance and recurrent urinary tract infections. Blood cell counts rapidly deteriorated during these infections followed by a slow recovery. Prednisone was gradually decreased and was discontinued in 1965. A few months later the androgens were discontinued too. Bone marrow investigations during the follow-up period are shown in table II.

Table II. Histological and cytological aspects of bone marrow investigations between May 1963 and February 1975 in patient II.

Month/year	May 1963	June 1963	August 1963	February 1964	March 1964	August 1964	November 1965	August 1974	February 1975
Cellularity	Hypocellular	More hypocellular	Diminished cellularity	Severely aplastic	Hypocellular	Nearly acellular	Normal cellularity	Increased cellularity	Increased cellularity
Blast cells	< 5%	< 5%	< 5%	< 5%	< 5%	< 5%	< 5%	15%	30-40%
Myelopoiesis	Diminished	Diminished	Sporadic	Absent	Diminished	Absent	Normal	Abnormal	Abnormal
Erytropoiesis	Diminished	Diminished	Normal	Absent	Diminished	Absent	Diminished	Diminished	Diminished
Mega-karyocytes	Diminished	Diminished	Diminished	Absent	Diminished	Absent	Normal	Diminished	Diminished
Lymphocytes	Normal/ Elevated	Normal	Normal	Diminished	Diminished	Absent	Normal	Diminished	Diminished

Without hematological treatment blood cell counts were normal till 1974. In August 1974 she was admitted because of fever. Liver, spleen and lymph nodes were not enlarged. The hemoglobin value was 11.5 g/100 ml, platelets 44,5 × 10^9/l and white cell count 16,6 × 10^9/l. The development of the blood cell counts is shown in figure 2. The differential count showed 64% immature blast forms. Bone marrow aspirate showed a hypercellular marrow, with few megakaryocytes, a normal but decreased erytropoiesis and

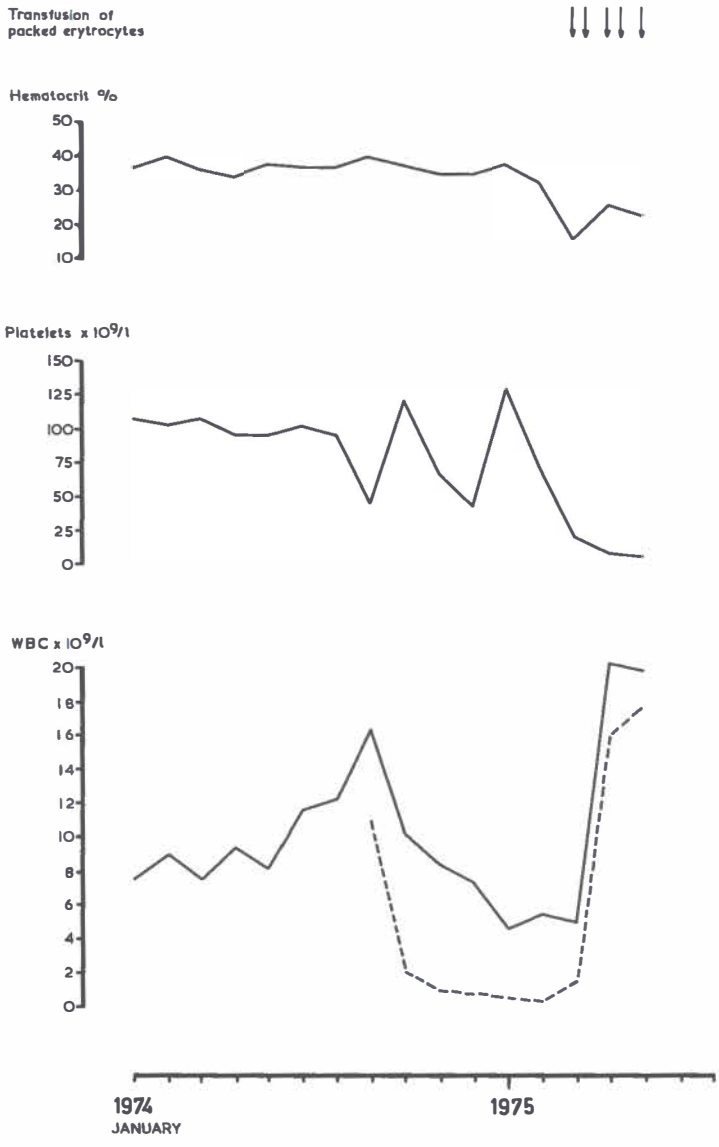

Figure 2. Course in hematocrit, platelets, WBC and leukemic blasts (----) during the terminal phase of the disease.

an excess of blasts. The blast cells often had a folded nucleus with fine chromatin structure and with one or more nucleoli. There was a substantial amount of grey-blue cytoplasm in the Giemsa-stain, without Auer rods. Cytochemistry is shown in table I. During the hospitalization the white blood cells spontaneously decreased till $6{,}9 \times 10^9/l$ with 5% immature blast cells. In the following months without therapy the white cell counts remained normal ($2 - 6 \times 10^9/l$) but the differential count showed an increase of blast cells. In March 1975 the patient was hospitalized again because of fever. The liver and lymph nodes were not enlarged, but the spleen was palpable 8 cm below the left costal margin and a few ecchymoses were present. The hemoglobin level was 4.6 g/100 ml, platelets $2 \times 10^9/l$ and white cell counts $2 \times 10^9/l$ with 30% blasts. Bone marrow aspiration and a biopsy showed hypercellular marrow almost completely replaced by blasts. There was a rapid increase in the white blood cell count and an increase in blast cells in the differential count. The patient died in April 1975, probably from a cerebral hemorrhage, before the therapy had been initiated. Autopsy was not performed.

DISCUSSION

In large series of patients with acquired aplastic anemia acute leukemia as a terminal event has not been recorded. There seems to be no obligatory link between these two diseases. Sporadic cases of aplastic anemia terminating in acute leukemia have however been described. In some of these cases aplasia does not seem to be an independent event as leukemia follows very rapidly after diagnosis of aplastic anemia (13). This group of patients should better be considered apart from those cases in whom aplasia persists for at least two years before leukemia develops (11). Adhering to this criterium Bloomfield and Brunning (4) found 125-150 cases reported in the last 20 years. The majority of the patients developing acute leukemia had been in contact with benzene. They found one case of idiopathic aplastic anemia, 13 cases of chloramphenicol associated aplasia and one case of aplastic anemia induced by phenylbutazone, terminating in acute leukemia. Alter et al reported the development of acute leukemia in three patients with idiopathic aplastic anemia in their series of 94 patients (2). Very little is known about the causal links between the different developments occurring from the very beginning of aplastic anemia to the eventually terminal leukemic phase. The occurrence of chromosomal abnormalities in bone marrow cells

at different levels of these processes is one of the intriguing aspects of this process. Benzene (8) and phenylbutazone (15) for example are known to induce chromosomal abnormalities in blood cells of exposed persons. But chromosome abnormalities belong also to the basic symptomatology of Fanconi's anemia, a disease due to an autosomal recessive gene mutation (6). On the other hand aplastic anemia as a possible consequence of exposition to benzene and phenylbutazone may terminate in leukemia as well as Fanconi's anemia. Nevertheless and in spite of the fact that some chromosomal abnormalities are recurring with relatively high frequencies in these affections there is no conclusive evidence pointing to a direct implication of chromosomal abnormalities in the development of these cases of leukemia. Furthermore in idiopathic aplasia (3, 10) the presence in itself of chromosomal abnormalities does not necessarily lead to the development of leukemia. However according to Rowley (12) chromosomal analysis of bone marrow cells may prove useful in guiding the clinician in the prognosis and therapy of leukemic disorders. So, as far as we know a relatively small number of drugs or chemicals seems to be involved in the development of leukemia. An altered immune system, related to the aplastic state (14) or resulting of therapeutic measures as the application of steroids (patient 11), may be of some importance in the development of leukemia. No relationship seems to be present between androgen therapy and the onset of leukemia (9), although the association between androgen treatment and liver tumors seems well established (7).

It is difficult to establish the cause of aplasia in patient 11. Abuse of acetylsalicylic acid has been reported to be associated with aplastic anemia (17). This would however be the first case described to be associated with the development of leukemia.

To establish which forms of aplastic anemia have a predisposition to the development of acute leukemia, especially acute non-lymphoblastic leukemia, it seems to be important to report all relevant cases.

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CHAPTER III

THE VALUE OF PROGNOSTIC INDICES IN APLASTIC ANAEMIA

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ZUSAMMENFASSUNG

Im Hinblick auf die Prognose wurde an 43 Patienten mit aplastischer Anämie die Zuverlässigkeit einiger Untersuchungsmethoden geprüft.

Mit Hilfe des Lynch-Index konnte bei 60% der Patienten, die innerhalb von 6 Monaten nach Abklärung der Diagnose starben, eine richtige Prognose gestellt werden; die Sensitivität war hierbei 82%. Mit dem Najean-Index konnte bei 40% dieser Patienten eine richtige Prognose gestellt werden, dieser Index ergab einer Sensitivität von 100%.

Die von Camitta und anderen ausgearbeiteten prognostischen Kriteria ergaben ein besseres Resultat: Bei 85% der Patienten war die mit Hilfe dieser Kriteria gesteltte Prognose richtig, d.h. in 15% der Fälle wurde zu Unrecht eine kürzere Lebensdauer vermutet; dies bei einer Sensitivität von 100%.

Der Lohrmann-Index, welcher sich auf die Retikulozytenanzahl als Gradmesser für die Prognose bezieht, ergab in dieser Serie eine Spezifizität von 64%.

Keine dieser Untersuchungsmethoden ermöglicht es bei Patienten mit einer längeren Lebensdauer, eine richtige Prognose zu stellen.

Zu diesem Zeitpunkt ist u.E. die Bestimmung der Leukozyten und Thrombozyten 3 Monate nach der Abklärung der Diagnose die Method der Wahl, um Patienten mit längerer (über 5 Jahren) und kürzerer Lebensdauer (unterhalb von 5 Jahren) prognostisch voneinander zu unterscheiden. Eine gleichzeitige Abnahme der Leukozyten und Thrombozyten ($>$ 10%) bedeutete bei allen Patienten den Tob innerhalb von 5 Jahren; Gleichbleiben oder Zunahme hingegen ergab bei 75% der Patienten eine günstigere Ueberlebensfrist.

SUMMARY

In 43 patients with aplastic anaemia we assessed the accuracy of different prognostic systems. Patients dying within 6 months after diagnosis were correctly predicted in 60% of cases with the Lynch-index with a sensitivity of 82%. With the Najean-index 40% of these patients are correctly predicted, this index has a sensitivity of 100%. More accurate are the prognostic criteria proposed by Camitta e.a. With these criteria, this rapidly fatal group is correctly predicted in 85% of the patients, indicating that 15% of patients are incorrectly predicted to have a limited survival, the sensitivity, however, is 100%. The Lohrmann-index, based on reticulocyte count predicts 64% of this group with severe aplasia.

Non of these prognostic systems do accurately predict long survival. We suggest that the best differentiation between patients with a long term prognosis (more than 5 years) and patients who die from aplastic anaemia within 5 years, is made by reevaluating the leukocyte and platelet count 3 months after the initial diagnosis. Decrease in blood counts (over 10%) predicts death from aplastic anaemia within 5 years correctly in all patients; stable or increased blood counts predict long survival in 75% of patients.

INTRODUCTION

The prognosis of aplastic anaemia is very variable. In acute, severe aplasia the patient may die within a few months after initial diagnosis. On the other hand he may survive for many years when a moderate, but persistent pancytopenia is present. A third group of patients will present with peripheral blood cell counts not compatible with acute severe aplastic anaemia, but these patients nevertheless die of their disease after a varying period of time during which deterioration of the blood cell counts occurs (5, 6, 11, 13, 14, 16, 25, 27).

The recent increase in therapeutic modalities in aplastic anaemia (1, 3, 8, 19, 20, 21, 23) has resulted in the problem of selecting the appropriate approach. While in acute severe aplasia, aggressive therapy is considered acceptable, patients with stable disease are probably best left alone.

Selection of the therapeutic regimen requires reliable prediction of prognosis at the earliest possible moment after diagnosis, and therefore increasing attention has been paid to prognostic factors in aplastic anaemia. We studied the value of different prognostic systems in our patient material with special attention to the group of patients with slowly deteriorating disease.

PATIENTS AND METHODS

Forty-three patients with pancytopenia and a hypo- or aplastic bone marrow were analysed. Peripheral blood cell counts were done by routine laboratory methods and were obtained at least monthly. Reticulocyte counts were corrected for haematocrit (normal: 48% in men, and 41% in women). Bone marrow was obtained by aspiration and by needle biopsy from the iliac crest. Peripheral blood pancytopenia was diagnosed when the haemoglobin level was below 12 g%, polymorphonuclear leukocytes below 2500/mm^3 and platelets below 120.000/mm^3 at the same moment. The diagnosis of aplastic anaemia was made on the presence of pancytopenia and a hypo- or aplastic marrow without evidence of malignancy, extensive fibrosis or storage disease. Screening tests to detect underlying diseases included liver functions, sugar water haemolysis, skeletal films and liver and spleen scans. From the personal history data were obtained about exposure to drugs and other toxic substances that might be associated with aplastic anaemia. Patients considered to have severe aplastic anaemia had less than 500/mm^3 granulocytes and less than 20.000/mm^3 platelets (3).

Patients were grouped according to the prognostic features as described by Camitta e.a. (5), Lohrmann e.a. (15), Lynch e.a. (16) and Najean e.a. (17) and the predicted survival according to these systems was compared to the actual survival of the patients.

As far as the actual survival is concerned, patients are divided into three groups

I. those with an actual survival for less than 6 months,
II. those who survive for more than 6 months but less than 5 years,
III. those who survive for more than 5 years.

In group II and III the haematological status after 3-4 months is compared with the situation at diagnosis (peripheral blood cell counts, bone marrow smears).

The prognostic criteria in the different index systems include the following parameters: index proposed by Camitta e.a.: Patients presenting with less than 500 polymorphonuclear neutrophils/mm^3, platelets less than 20.000/mm^3 and a corrected reticulocyte count less than 1% have a probability of survival for more than 6 months after diagnosis of less than 20% (5). Index proposed by Lohrmann e.a.: Patients presenting with less than 10.000 reticulocytes/mm^3 (correlating with a reticulocyte count of 0.28%) have a probability to survive for more than 6 months of less than 50% (15). Index

proposed by Lynch e.a.: It is calculated from the following prognostic formula (16): C = 0.01796 (B) + 0.01271 (S) — 0.00008 (OFV) — 0.00359 (R) — 0.000002 (N) — 0.00018 (P) + 0.00046 (NM), where C is the prognostic index (unitless). B refers to onset with bleeding; it was graded 0 if bleeding was present at the onset of symptoms and graded 1 if bleeding was absent. S (sex) was graded 1 if the patient was female, 2 if the patient was male. OFV is the interval between onset of symptoms and first clinic visit in months. R is the corrected initial reticulocyte count (per cent). N is the initial neutrophil concentration (cells/mm^3). P is the initial platelet concentration (thousands/mm^3). NM is the per cent nonmyeloid cells in the initial marrow aspirate. All patients scoring more than 0.041 at diagnosis have a survival less than 4 months, while all patients scoring an index below 0.000 will survive for more than 4 months; 60% of the patients with an index between 0.033 and 0.041 will die within 4 months, compared to 24% of the patients with a score between 0.000 and 0.033.

Najean e.a. calculated a discriminant linear function: 0.544 A + 0.027 B — 0.416 C — 0.209 D + 0.024 E + 0.134 F + 0.053 G, where A = percentage of the myeloid cells in the bone marrow; B = reticulocyte count (10^9/litre); C = haemorrhagic symptoms (1 = absent, 2 = moderate, 3 = severe); D = sex (1 = male, 2 = female); E = granulocytes (10^6/litre); F = platelet count (10^9/litre); G = delay between first symptoms and admission (months). Of the patients with a mildly severe aplastic anaemia (index higher than 50) 83% were correctly classified as patients with a survival longer than 6 months. Only 50% of the patients, with an index lower than 50, which should correlate with a short survival, were correctly classified (17).

Patients who required regular bloodtransfusions were treated with oxymetholone 60 mg daily. This therapy was continued for at least 3 months. When improvement had not occurred medication was stopped.

RESULTS

Patients were divided into three groups according to survival (Table I).

Group I: patients surviving for less than 6 months (patients 1-11).

Group II. patients surviving for 6 months but dying within 5 years from aplastic anaemia (patients 12-28).

Group III: patients who survived for more than 5 years following diagnosis of aplastic anaemia (patients 29-43).

To these groups were applied the prognostic criteria according to Lynch e.a. (16), Najean e.a. (17), Camitta e.a. (5) and Lohrmann e.a. (15). Using the

Table I. Characteristics of 43 patients with aplastic anaemia.

Pat. nr.	Age years	Sex	Aetiology	Hb g%	Ret. %	P.M.N. /mm³	Plat. /mm³	Lynch class.	Najean index	Survival months
1	16	M	Idiopathic	6.0	0.08	110	6.000	+ 0.052	20	1
2	56	M	Antipyrin	4.9	0.35	140	12.000	+ 0.049	26	6
3	62	M	Idiopathic	7.5	0.04	20	2.000	+ 0.060	12	1
4	34	M	Idiopathic	8.2	0.11	20	2.500	+ 0.055	17	3
5	22	M	Idiopathic	7.8	0.49	340	10.000	+ 0.057	12	2
6	16	M	Idiopathic	7.9	0.28	220	11.000	+ 0.063	6	2
7	63	M	Idiopathic	7.6	0.04	20	12.000	+ 0.059	18	1
8	14	F	Idiopathic	6.8	0.97	260	2.000	+ 0.051	5	4
9	17	F	Butazolidin	7.9	0.85	10	3.000	—0.037	6	1
10	68	F	Orphenadrin	4.6	0.01	80	16.000	+ 0.021	38	1
11	52	F	Idiopathic	8.9	0.19	10	1.000	+ 0.037	24	1
12	60	M	Idiopathic	9.1	1.16	460	27.000	+ 0.006	53	26½
13	21	F	Sulfa	6.3	0.19	2300	14.000	+ 0.036	18	14
14	61	M	Butazolidin	4.7	1.18	1700	77.500	+ 0.025	56	9
15	75	M	Idiopathic	11.4	0.57	850	58.000	+ 0.025	50	18
16	69	M	Idiopathic	5.8	1.97	800	55.000	+ 0.011	58	14
17	68	M	Idiopathic	11.0	0.80	600	48.000	+ 0.035	40	25
18	63	M	Idiopathic	9.6	1.37	780	85.000	+ 0.007	60	22
19	73	M	Idiopathic	6.8	0.39	1200	103.000	—0.001	54	10
20	62	M	Idiopathic	5.0	0.25	200	30.000	+ 0.008	52	21
21	38	F	Idiopathic	4.8	0.12	220	80.000	—0.008	50	23
22	23	M	Idiopathic	9.2	2.04	1000	98.000	+ 0.040	21	37
23	18	F	Idiopathic	9.5	0.39	2400	5.000	—0.010	50	15½
24	42	M	Lindaan	7.7	11.9	1550	7.000	+ 0.032	47	8½
25	69	M	Idiopathic	12.7	0.61	280	37.000	+ 0.021	53	20
26	61	M	Nivaquine	10.6	2.47	550	52.000	—0.008	59	14
27	75	F	Gold	10.0	3.00	1400	37.000	+ 0.011	55	49
28	25	M	Idiopathic	11.3	1.83	1900	71.000	—0.011	63	36
29	32	F	Idiopathic	8.0	0.60	900	2.500	—0.091	13	74+
30	63	M	Buta + CAP*	4.2	0.20	1250	1.800	+ 0.055	15	105+
31	16	F	Idiopathic	7.9	1.22	1250	20.000	—0.012	53	65+
32	20	M	Idiopathic	4.9	0.37	600	10.000	+ 0.013	43	92
33	70	F	Plaquenil	8.7	1.70	2150	55.000	—0.001	54	120
34	23	F	Idiopathic	10.0	6.58	900	85.000	—0.016	53	120+
35	63	M	CAP*	3.5	0.32	2500	9.000	+ 0.024	43	60+
36	31	M	Tolbutamide	10.8	0.68	2100	32.000	+ 0.001	51	100+
37	22	F	Sulfa	7.5	3.68	500	21.000	—0.003	49	61+
38	35	M	Idiopathic	9.7	1.32	1750	25.000	—0.003	48	72+
39	18	M	Idiopathic	8.7	2.49	1300	13.000	+ 0.026	46	59+
40	29	M	Idiopathic	7.8	1.28	2300	22.000	+ 0.025	22	132+
41	47	M	Idiopathic	9.5	0.56	100	25.000	+ 0.035	40	120+
42	48	F	Idiopathic	7.4	0.79	100	15.000	+ 0.013	51	96+
43	37	M	Idiopathic	5.4	0.45	430	12.500	+ 0.040	12	89

* CAP = Chlooramphenicol

prognostic criteria of Lynch e.a. (16) for patients who had a survival of less than about 6 months, we were able to make a correct prediction in 60 per cent of these patients, with a sensitivity of 82 per cent. With the Najean-index these values were 40 per cent and 100 per cent. Using the criteria for severe anaemia proposed by Camitta e.a. (5) a rapidly fatal outcome could

correctly be predicted in 85 per cent, with a sensitivity of 100 per cent, which means that all patients with a rapidly fatal outcome were predicted correctly. When the number of reticulocytes less than 0.28% was correlated with a short survival (15), 64 per cent were predicted correctly with a sensitivity of 64 per cent. Patients with a survival of more than five years were predicted with a Lynch index value below 0.000 (16) in 50 per cent with a sensitivity of 40%; using the Camitta criteria of non-severe aplastic anaemia (5) 43 per cent were predicted correctly with a sensitivity of 80%; with the Lohrmann criteria (15) with reticulocytes more than 0.28% 44 per cent of the patients who survive more than five years were correctly predicted with a sensitivity of 93%. We could not find a value of the Najean-index to identify these patients. To identify the patients with an intermediate survival more than six months but less than five years, we used the prognostic index of Lynch with values between 0.000 and 0.041 (16), the prognostic criteria for non-severe aplastic anaemia of Camitta (5) and the reticulocyte count >0.28% of Lohrmann (15). The corresponding percentages for a correct prediction are 54%, 57% and 44%; the sensitivity values are 70%, 100% and 82% (Table II).

Table II. Value of four prognostic systems in 43 patients with aplastic anaemia.

Survival	Prognostic classification	Correctly predicted	Sensitivity
⩽ 6 months	Lynch e.a.: > 0.033	9/15 = 60%	9/11 = 82%
	Najean e.a.: ⩽ 50	11/28 = 40%	11/11 = 100%
	Camitta e.a.: Severe aplastic anaemia	11/13 = 85%	11/11 = 100%
	Lohrmann e.a.: High risk patients	7/11 = 64%	7/11 = 64%
> 6 months	Najean e.a.: > 50	15/15 = 100%	15/32 = 56%
> 6 months and < 5 years	Lynch e.a.: 0.000-0.041	12/22 = 54%	12/17 = 70%
	Camitta e.a.: Non-severe aplastic anaemia	17/30 = 57%	17/17 = 100%
	Lohrmann e.a.: Low risk patients	14/32 = 44%	14/17 = 82%
> 5 years	Lynch e.a.: < 0.000	6/12 = 50%	6/15 = 40%
	Camitta e.a.: Non-severe aplastic anaemia	13/30 = 43%	13/15 = 80%
	Lohrmann e.a.: Low risk patients	14/32 = 44%	14/15 = 93%

In the patients from group II and III polymorphonuclear leukocytes and platelets were reevaluated at 3 months (Table III) (Mean of three values are given). An increase in granulocytes and/or platelets was considered to represent improvement; deteriorating disease was present if both granulocytes and platelets decreased more than 10% compared to initial values. Looking at decreasing blood cell counts we were able to identify correctly

Table III. Increase - decrease of P.M.N. and platelets after three months observation compared with initial values.

Pat. nr.	P.M.N./mm³	Platelets/mm³
12	— 200	— 10.000
13	— 1950	— 6.500
14	— 1400	— 65.000
15	— 100	— 32.000
16	— 200	— 45.000
17	— 300	— 23.000
18	— 250	— 55.000
19	— 500	— 17.000
20	— 100	— 15.000
21	— 50	— 44.000
22	— 800	— 48.000
23	— 1400	— 4.500
24	+ 200	+ 26.000
25	+ 400	+ 7.000
26	— 500	+ 2.000
27	— 1000	— 35.000
28	— 1100	+ 16.000
29	— 300	+ 2.500
30	— 150	+ 2.000
31	+ 300	+ 0
32	— 50	+ 25.000
33	+ 550	+ 49.000
34	+ 300	+ 25.000
35	+ 1200	+ 8.000
36	— 300	+ 60.000
37	+ 1150	+ 9.000
38	+ 500	+ 35.000
39	+ 500	+ 9.500
40	+ 500	+ 3.000
41	+ 1100	+ 15.000
42	+ 500	+ 20.000
43	+ 1500	+ 55.000

Table IV. Prognostic value of increasing or decreasing blood cells after three months compared with initial values.

Survival	Blood cells	Correctly predicted	Sensitivity
> 6 months < 5 years	Decreasing	13/13 = 100%	13/17 = 80%
> 5 years	Increasing	15/19 = 75%	15/15 = 100%

those patients with an intermediate survival in 100% and a sensitivity of 80%. When increasing blood cells are correlated with a survival of more than five years, the patients with this favourable outcome were detected in 75 per cent correctly and a sensitivity of 100% (Table IV).

DISCUSSION

Many attempts have been described to predict the survival of patients with aplastic anaemia early in the disease, but the results are confusing. Haak e.a. (9) correlated a poor prognosis with drug-induced disease, Lewis (13) with an onset after 40 years of age. Both factors are not discriminating in the study of Vincent and De Gruchy (25). A bleeding tendency at admission (16, 25) and low granulocyte and platelet counts (5, 6, 13) seem to be associated with a limited life prognosis, however the value of the low granulocyte and platelet counts is debated by Vincent and De Gruchy (25) and Lohrmann e.a. (15). Bone marrow cellularity has been found to influence survival in some (10), but not in all studies (6, 9, 12, 25). Marked lymphocytic (7, 14) or inflammatory infiltration (22) has been described to correlate with a poor prognosis. Isotope studies can be of some use in the prediction of prognosis, but their value is limited because of methodological problems (4) and difficulties in reproducibility of quantitation (18).

In contradiction to the results of Van Hengstum e.a. (24) who did not find a difference in survival between patients classified as having severe or non-severe aplastic anaemia, in our experience all patients with a survival less than 6 months were predicted by the criteria of severe aplastic anaemia as described by Camitta e.a. (5). However, two patients (no. 42 and 43, table I) were predicted by the Camitta criteria to have a short survival, but they lived for 96 and 89 months respectively. This percentage of false positives is in accordance with the publication of Camitta e.a. This finding, however, is not to be neglected as 15% of the patients classified as short survivors may have a rather favourable prognosis in a fair clinical condition, that would not benefit from aggressive therapy. In predicting a short survival the Camitta criteria are more discriminating than the reticulocyte counts as described by Lohrmann e.a. (15). Both have the value of reproducibility and objectivity over the Lynch-index (16) and Najean-index (17) in which the difficult to evaluate number of non-myeloid cells in the bone marrow smear is an important factor. Furthermore, sampling an aplastic marrow by aspiration or biopsy can result in an unrepresentative quantification because of the variability of marrow cellularity in aplastic anaemia (7). In our hand the

Lynch-index (16) as well as the index described by Najean e.a. (17) have less value in predicting a survival less than 6 months. In a recent study, the Lynch-index had no more predictive value than the clinical impression (22). In children, the Lynch-index was predictive only in those patients with a rapidly fatal course (26). Lohrmann e.a. (15) found a limit of 10.000 reticulocytes/mm^3 at the time of diagnosis of severe aplastic anaemia to be of great value in identifying an extremely poor prognosis in patients under 45 years of age. We could not make a good distinction between the different survival groups using this criterium as a single parameter. However, this parameter was applied to the whole patient group including patients over 45 years of age. Evaluating the reticulocyte criterium, Van Hengstum e.a. (24) found no difference in survival between patients with less and with more than 10.000 reticulocytes/mm^3 at admission, they also included patients older than 45 years of age.

In patients with an intermediate survival 6 months - 5 years additional information concerning the eventual outcome has been sought in follow-up studies. Lynch found no changes in his prognostic index-calculations during follow-up (16). Te Velde and Haak (22) showed the value of repeated bone marrow biopsies, Speck e.a. (20) evaluated the reticulocyte and granulocyte counts 8 weeks after diagnosis and Haak e.a. (9) found a bad prognosis in patients whose granulocyte count remains under 1.000/mm^3 and platelet count under 20.000/mm^3 6 months after diagnosis. In agreement with Camitta e.a. (5) we find in 13 out of 32 patients initially presenting with a mild aplastic anaemia deterioration to a lower level of granulocytes and platelets to a level compatible with severe aplasia. These patients will die of aplastic anaemia and they have a limited life prognosis in comparison to patients with stable disease. Therefore it seems important to predict this survival pattern as soon as possible after diagnosis. In our patients the occurrence of chronic progressive disease leading to death could be predicted by a decrease in both granulocyte and platelet counts three months after diagnosis in all 13 patients. This is in accordance with the recently published study of Najean e.a. (17). However, patients may die of aplastic anaemia without showing deterioration in both cellines at 3 months (patient no. 24, 25, 26 and 28). Patient 24 was unfortunately re-exposed to the agent causing the initial aplasia six months after diagnosis. The advanced age of patient 25 and 26 may indicate that other factors than aplasia were important in the clinical course.

The recognition of the survival pattern of patients in an early stage of

their disease may have clinical consequences. The continuing deterioration of the blood cell counts in the group with progressive disease might indicate the persistence of a continuing bone marrow insult. Some of these can be eliminated, which means that especially in the group of patients with progressive disease drugs should be avoided as much as possible and a careful search for infectious foci may be worthwhile. Continuous aggression towards the bone marrow could also be the result of autoimmune fenomena. Evidence of this situation is thought to be expressed by the finding of lymphoid infiltration in the bone marrow (2).

In summary we conclude that severe aplasia with a short survival can be predicted at the moment of diagnosis when levels of reticulocytes, granulocytes and platelets are low. Mild aplasia can lead to a constant stable disease and this can be predicted when no deterioration of the blood cell counts is present after three months. On the other hand when at this moment the blood cell counts are decreased the patients will probably eventually die of aplastic anaemia. The early recognition of the survival pattern in the individual patient may have important consequences as to the choice of therapy and the diagnostic approach. Aggressive therapy may be justified in acute severe aplastic anaemia, patients with chronic stable disease should be treated symptomatically. Several ways of treatment may be indicated in patients with chronic progressive disease, some of these patients may benefit from androgens but immunosuppressive therapy may be indicated when there is evidence of immunological aggression against the bone marrow.

Further studies into the cause of deterioration of bone marrow function in these patients may shed new light on the mechanism leading to aplasia.

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CHAPTER IV

INFECTION PREVENTION IN GRANULOCYTOPENIC PATIENTS BY SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT

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ABSTRACT

In a controlled prospective randomized trial we studied the effect of selective decontamination of the digestive tract (SDD) in granulocytopenic patients on the frequency of infections. By SDD it was aimed to suppress the pathogenic Gram-negative micro-organisms and yeasts without affecting the non-pathogenic anaerobic flora. This anaerobic flora was maintained intact because of its value for the colonization resistance of the gastrointestinal tract.

SDD was accomplished by oral administration of nalidixic acid or cotrimoxazole or polymyxin E to suppress growth of aerobic Gram-negative bacteria, and amphotericin-B to inhibit growth of yeasts.

Gram-negative or yeast infections occurred in the control group 18 times in 12 patients; in the decontaminated group two times in two patients ($P<0.01$). Clinical infections occurred 15 times in 12 control patients and four times in three SDD treated patients ($0.01<P<0.05$). While nine patients in the control group died with an acquired infection none died in the SDD treated group ($P<0.01$).

It is concluded that SDD is a promising and widely applicable method of infection prevention. It decreases the need for treatment in a "protected environment".

INTRODUCTION

Prolonged periods of neutropenia frequently occur in patients with aplastic anemia or with acute leukemia, either as a manifestation of the

disease or of its treatment. Both the number of granulocytes and the duration of the neutropenia are related to the incidence and severity of infections (1-4). The bacterial infections are predominantly due to Gram-negative rods (2, 4-10), which may in most cases find their port of entry in the patients own gastrointestinal tract (7, 9, 11, 12). However, yeasts and fungi may also cause severe infections in these patients (13). Therefore, infection prevention has been attempted in neutropenic patients by "sterilization" of the gut either inside or outside a "protected environment" (3, 9, 11). For sterilization of the digestive tract one or more of the following oral non-absorbable antibiotics have been used: gentamicin, vancomycin, cephaloridin and neomycin. The dosages of these oral antibiotics are such that not only the potentially pathogenic microorganisms but also the non-pathogenic anaerobic part of the flora is suppressed or eliminated (14-17). In animal as well as human studies this anaerobic flora has been shown to limit colonization of the digestive tract by aerobic potentially pathogenic species and to prevent overgrowth by such microorganisms (18). The potentially pathogenic Gram-negative flora of the digestive tract (*Pseudomonas aeruginosa,* Escherichiae, Citrobacter, Klebsiellae and Proteae) is not static in composition. Under normal conditions different species contaminate the digestive tract continuously by oral route (19, 20). However, most of these contaminating potentially pathogenic microorganisms colonize the g.i.-tract for short periods in low concentrations; very few can colonize, i.c. persist longer (several months). Certain species of the anaerobic flora constitute a natural barrier against newly acquired pathogenic bacteria in a manner as yet only partially understood. This barrier has been called colonization resistance (21, 22).

The above mentioned antimicrobial drugs for "gut-sterilization" all lead to suppression of these "protective" anaerobic bacterial species and thereby of the colonization resistance.

A decrease of the colonization resistance considerably diminishes the threshold dose for colonization by aerobic potentially pathogenic species. This makes protective isolation necessary, particularly in a hospital environment in which resistant strains may exist.

Those drugs which suppress or eliminate potentially pathogenic microorganisms from the g.i.-tract lumen without affecting the CR-constituting anaerobic flora would leave the colonization resistance intact. We have used such drugs for SDD in neutropenic patients. Originally developed in experimental animals (23), this approach was followed in a prospectively ran-

domized controlled study in which nalidixic acid, co-trimoxazole or polymyxin E were given either alone or in combination, but in all cases together with amphotericin-B. These antimicrobial agents in the dosages used do not affect anaerobic bacteria but can suppress growth of Enterobacteriaceae, Pseudomonodaceae and *Candida* species (24-33).

MATERIALS AND METHODS

Patients

In this prospective, randomized controlled trial, all consecutive adult patients with granulocytopenia due to bone marrow failure, acute myeloid leukemia, acute non-myeloid leukemia, or their treatment, and who were hospitalized between 01-01-1977 and 10-15-1978, were admitted to the study as soon as their peripheral granulocyte count had decreased below 1000 per mm^3. Patients were randomized to receive either oral antibiotics aimed at selective decontamination of the digestive tract (SDD) or to serve as a control group. The patients were stratified into the following three diagnostic groups in order to obtain an even distribution of these diagnoses over the SDD and the control group: aplastic anemia, acute myeloid leukemia and acute non-myeloid leukemia.

Some patients were hospitalized more than once, and each hospitalization period was considered to be a separate case.

The study period of a case was terminated upon discharge from the hospital, when three times in succession the granulocyte count was above 1000 per mm^3, or at death. Cases studied for less than 7 days, or in whom the protocol was not correctly followed, were excluded from the study.

After randomization, the choice of the antibiotics used for selective decontamination was based on the results of the microbiological surveillance and on known or presumed hypersensitivity.

Microbiological surveillance

In both the selectively decontaminated and control group, cultures from the throat and the feces were performed three times per week. Cultures were made from stools or occasionally from an anal swab.

Solely an aerobic culture was made on the specimens, with special attention given to potential pathogens such as aerobic Gram-negative rods (MacConkey agar, Merck), yeasts and fungi (Sabourad agar, Merck),

Staphylococci and Streptococci (blood agar, Oxoid) and *Haemophilus influenzae* (Levinthal agar, Oxoid).

Gram-negative rods were identified and biotyped with A.P.I. 20 E-system (A.P.I. System S.A. La Balme Les Grottes, France) (12, 34, 35).

From all different biotypes the sensitivity pattern was determined by a standard series of antibiotics currently in use for the treatment of infections. In addition the sensitivity of nalidixic acid, co-trimoxazole and polymyxin E was determined.

Surveillance of the colonization resistance

Contamination experiments with antibiotic resistant Enterobacteriaceae showed that anaerobes are responsible for the difference in the "threshold contamination dose for colonization" between conventional and antibiotic-treated or germ-free mice (21, 22). In other experiments it was shown that mice colonized with exclusively aerobic bacteria isolated from conventional mice had no resistance to colonization with resistant Enterobacteriaceae, while human anaerobes caused an increase in the C.R. of the gastrointestinal tract in animals as well as in man (18). Also, it was found that nalidixic acid (23), co-trimoxazole and polymyxin did not influence the C.R.

The C.R. during antibiotic therapy can be directly expressed as the log concentration of a specific resistant microorganism (22). If the C.R. is decreased by a complete decontamination, only a few contaminating bacteria resistant to the antibiotics used become established in unusually high concentrations in the digestive tract (36), so that a decreased C.R. is reflected in an increased concentration of resistant microorganism.

During therapy with nalidixic acid, co-trimoxazole or polymyxin, the unaffected C.R. is expressed by a constant concentration of the naturally resistant enterococci in the stool (23). This observation was confirmed by others (37, 38).

Because the success in recovering anaerobes from the stool is largely determined by the care used in transportation and by laboratory facilities, and because isolation and identification of anaerobes are time-consuming (39), we decided to routinely use the concentration of the enterococci in the stool of the SDD patients as a practicable measurement of the colonization resistance, instead of culturing anaerobes. Wade e.a. (40), evaluating the use of co-trimoxazole - albeit in a slightly lower dose per day - as infection prophylaxis, however, found with extensive anaerobic cultures of the stool that the anaerobes were preserved, while the aerobes were suppressed.

Selective decontamination

As the goal of this trial was to suppress aerobic Gram-negative bacteria and yeasts to undetectable concentrations, the results of the microbiological surveillance determined the choice of the antimicrobial drugs used for SDD. Differences in the sensitivity pattern of the aerobic digestive tract flora and inevitably occurring side effects, such as allergy to an antimicrobial drug, required the availability of a number of different antibiotics. At the onset of the study nalidixic acid, co-trimoxazole, polymyxin E and amphotericin-B were available for this purpose. These antibiotics leave the colonization resistance intact, and the first three can be used interchangeably.

Selective decontamination of the digestive tract (SDD) for Gram-negative rods was consequently attained with nalidixic acid (8 g/day) or with co-trimoxazole (three times a day two tablets, each tablet containing 80 mg trimethoprim and 400 mg sulfamethoxazole) or with polymyxin E (800 mg/day). For selective elimination of yeasts, an amphotericin-B suspension (2 g/day) was used. With exception of co-trimoxazole, the daily doses of the antimicrobial drugs were divided into four portions. All drugs were given orally.

Hematological surveillance

White blood cells were counted by the Coulter counter (>3000 cells per mm^3) or in a counting chamber (<3000 cells per mm^3).

Absolute levels of granulocytes were measured in the hemocytometer or calculated from a differential count and the total white blood cell count. Differential counting was performed on 300 cells in a buffy coat smear. Counting of granulocytes was repeated three times a week.

Clinical surveillance and treatment

Complete physical examination and radiographic examination of the chest and sinuses as well as microbiological investigations were performed in each patient on the day of randomization and repeated when the patient had fever. In case of a suspected infection, other specimens i.e., blood, urine and sputum were cultured. When axillary temperature rose above 38.5°C blood cultures were performed.

All patients, the selectively decontaminated as well as the control group, were treated in conventional four-bed hospital rooms under standard conditions. Standard non-sterile hospital food was provided. The patients of

both groups were treated by the same group of physicians. Patients with acute non-myeloid leukemia were treated with a regime consisting of vincristine, prednisone and 1-asparaginase. Daunorubicin was added when indicated. Acute myeloid leukemia was treated with a regime consisting of arabinosyl cytosine, 6-thioguanine and daunorubicin (41) or with a less aggressive therapy consisting of lower dosages of the same drugs. When indicated, supportive treatment was given. This included R.B.C., granulocytes or platelet transfusions as well as i.v. supply of broadspectrum bactericidal antibiotics (gentamicin + carbenicillin) and local or systemic antimycotic therapy. Local antimycotic therapy consisted of oral application of amphotericin-B in orabase (R) (42), or lozenges.

Registration of acquired infections

Fever day. Registered when axillary temperature was above 38.5°C.

Microbiologically documented infection. Defined as the presence of definite signs and symptoms of infection plus the isolation and identification of pathogenic microorganisms from blood, urine, sputum or local sites.

Clinically documented infection. Defined as the presence of definite signs and symptoms of infection with negative cultures.

Non-infectious, "allergic" fever. Fever associated with a non-infectious cause such as blood transfusion, administration of antileukemic therapy or with an allergic reaction to drugs.

Fever of unknown origin. Defined as a fever not associated with signs or symptoms of infection and without positive cultures or manifest allergic reactions.

Statistical analysis

The end-point of the trial was determined beforehand, so that the control group and the SDD-treated group would consist of at least 50 patients each. This number was chosen so that the comparison of the percentages of infected patients (α=0.05) could reveal a 75% reduction in the number of infected patients in the SDD group with 95% chance. Of the control group patients, 30-50% were expected to develop an infection; this expectation was based on previously treated patients and on data published by others (4-10).

When comparing the percentages of patients, the Fisher's exact test was used with α=0.05.

RESULTS

Patients

One hundred and thirteen cases were randomized and 105 were evaluable; 20 patients were hospitalized more than once. Eight patients were not evaluable because of death or discharge from the hospital within 7 days, or because the period of SDD was less than 7 days as a result of a short period of granulocytopenia.

Pertinent details concerning the evaluated patients are given in Table 1.

Table 1. Patient characteristics.

	Acute non-myeloid leukemia		Aplastic anemia		Acute myeloid leukemia		All diagnoses	
	SDD	control	SDD	control	SDD	control	SDD	control
Number of entrances	17	16	19	20	22	19	58	55
Excluded	1	1	1	1	3	1	5	3
Number of cases studied	16	15	18	19	19	18	53	52
Males	10	11	10	11	10	11	30	33
Females	6	4	8	8	9	7	23	19
Average age (yr)	37.2	29.7	42.9	53.5	52.5	53.3	44.6	46.6
Number of weeks of study	73.0	46.5	58.0	66.0	78.5	79.5	209.5	192.0
Weeks with granulocytes per mm³								
⩽ 100	13.0	11.0	21.5	21.5	38.0	42.0	72.5	74.5
101-500	33.0	18.5	20.0	20.5	21.5	22.5	74.5	61.5
>501 <1000	27.0	17.0	16.5	24.0	19.0	15.0	62.5	56.0
Number of cases with infection at admission	0	1	4	3	2	4	6	8

No important difference in the number of patients nor in the average age was found between the SDD- and the control group. In both groups the total number of weeks on study was comparable. This was also the case with the distribution of periods with granulocyte counts less than 100 per mm³ (35%, resp. 38%), between 100 and 500 per mm³ (35%, resp. 32%) and above 500 per mm³ (30%, resp. 30% of the weeks of study) among the subgroups.

Microbiological surveillance

Inventory. In all 105 cases Gram-negative rods were cultured from the feces on admission; in 26 cases these microorganisms were cultured from the throatswab (13 cases in the control group). In the 105 cases investigated on admission, the fecal flora was sensitive to nalidixic acid in 96.9%, to co-trimoxazole in 78.7% and to polymyxin E in 80.4%. No cases of micro-organisms resistant to all three antibiotics could be found.

Control patients. The aerobic part of the fecal population (Enterobacteriaceae, Pseudomonodaceae and *Candida* species) of the control patients was constantly changing during their hospital stay; this had also been reported by others (19, 20).

In 37 control patients Gram-negative rods were isolated from throat cultures.

Selective decontamination. From the 53 SDD patients, 32 started with nalidixic acid; successful decontamination (suppression of aerobic Gram-negative bacteria and yeasts to undetectable concentrations) was achieved after an average period of 6.9 days (S.D. 3.9 days). Eighteen patients started with co-trimoxazole, resulting in decontamination after 8.9 days (S.D. 4.6 days). In three patients who started with nalidixic acid, treatment had to be changed to co-trimoxazole within a couple of days because of nausea; they were decontaminated in 9.0 days (S.D. 1.7 days). None of the patients were initially treated with polymyxin E.

Fecal Gram-negative rods in SDD patients. Following successful decontamination, 16 patients remained completely free of potentially pathogenic Gram-negative rods (Enterobacteriaceae and Pseudomonodaceae) in their stool cultures. In 26 patients Gram-negative bacteria were occasionally found in low concentrations ($<10^3$ bacteria per gram feces) These microorganisms were often different in species and biotype from those cultured during the inventory period. Usually, these isolated Gram-negative bacteria were still sensitive to the drugs used for decontamination (this had been reported separately (43), and they disappeared within 2-3 days.

In 40% of the cases in which resistant microorganisms were isolated, they also disappeared without changing the antimicrobial therapy; in 60% the antimicrobial therapy was changed, promptly resulting in negative fecal cultures.

Oropharyngeal Gram-negative rods in SDD patients. Thirteen of the 53 decontaminated patients were found to have sporadically Gram-negative rods in their throat cultures.

Of the microorganisms isolated from the throat swabs in the SDD group, 70% was sensitive to the antibiotic used for SDD. Usually the contaminating agent disappeared without change of treatment.

Evaluation of the colonization resistance in SDD patients. Since we decided to use the concentration of the enterococci in the stool of the SDD patients as a measurement of the colonization resistance, the concentration of these microorganisms naturally resistant to the antimicrobial agents used was determined in each fecal culture.

During the selective decontamination, the concentration of the enterococci in the feces remained constant and did not differ from the concentration during the inventory period.

Infection prevention

As statistical analysis on the effect of SDD on the occurrence of infections is most appropriately done by comparing the number of infected patients, these data are therefore presented separately (Tables 2 and 3) from the data on occurrence of infections in both groups (sometimes more than one during the hospitalization of a patient) (Tables 4 and 5).

Table 2. The number of patients who experienced one or more infections during SDD in comparison with the control group of patients.

Type of infection	Number of patients		
	SDD	Control	*P*-value
All kinds of infections (F.U.O. included)	10	24	<0.01
Infections due to Gram-negative bacteria or yeasts	2	12	<0.01
Clinically documented infections	3	12	0.01-0.025

Table 3. Number and percentage of infected patients during various stages of granulocytopenia in the selectively decontaminated and control group.

Granulocyte count	Number and percentage of infected patients		
	SDD	Control	*P*-value
≤100	6 (26%)	14 (56%)	0.025-0.05
101-500	2 (6%)	11 (35%)	<0.01
>500	3 (10%)	5 (17%)	>0.10
Total	10(19%)	24 (46%)	<0.01

Table 4. The number of infections in SDD treated and in control patients.

	SDD	Control
Total number of infections	9	38
Infections due to Gram-negative bacteria or yeasts	2	18
Infections due to Gram-positive bacteria	3	5
Clinically documented infections	4	15
Episodes with F.U.O.	7	7

Table 5. Occurrence of episodes with infections and fever of unknown origin during various stages of granulocytopenia in selectively decontaminated and control patients.

	Microbiologically proven infections		Infectious episodes caused by Gram-neg. bacteria or yeasts		Clinical infectious episodes		Fever of unknown origin	
Leucocyte count	SDD	control	SDD	control	SDD	control	SDD	control
Aplastic anemia								
≤100	1	5	0	4	1	0	0	0
101-500	0	2	0	1	0	2	0	0
>500	0	1	0	1	0	0	0	0
Acute myeloid leukemia								
≤100	2	8	1	6	2	6	1	3
101-500	0	1	0	1	1	2	3	2
>500	0	1	0	1	0	2	1	0
Acute non-myeloid leukemia								
≤100	1	4	1	3	0	1	1	0
101-500	0	0	0	0	0	1	0	2
>500	1	1	0	1	0	1	1	0
All diagnoses								
≤100	4	17	2	13	3	7	2	3
101-500	0	3	0	2	1	5	3	4
>500	1	3	0	3	0	3	2	0
Total	5	23	2	18	4	15	7	7

Because selective decontamination was exclusively directed against Gram-negative rods and yeasts, it was only expected to be effective in preventing infections due to these microorganisms. However, patients treated with co-trimoxazole may have been protected against Gram-positive cocci, although they were not necessarily suppressed in the g.i.-tract by the drug. Nevertheless, an overall decrease in infection frequency of more than 75% was achieved by selective decontamination of the digestive tract (Table 4). Only one Gram-negative and one yeast infection occurred in the SDD patients. One of these patients developed cellulitis due to extravasation of infusion solution and this area became infected by *Acinetobacter calcoaceticus*. This microorganism was not cultured from the stool or the throat swab at the time of infection. The other patient who could not continue the antifungal part of the SDD medication because of nausea, developed a *Candida krusei* sepsis shortly after developing overgrowth in the gut with the same organism. The incidence of Gram-positive infections was similar in both the SDD and the control group (resp. 3 and 5 infections). Also, the number of clinically documented infections was reduced in the decontaminated patients.

Twelve patients in the control group and five in the SDD group developed Candida lesions ($P=0.051$). Nearly all occurred in the mouth and were successfully treated with local amphotericin-B.

Most infections occurred in the control patients in the period during which they had less than 100 granulocytes, especially in patients with acute leukemia. The decrease in the infection incidence in these patients by selective decontamination is remarkable (Table 5). However, not only the number of infections was reduced, but also the number of patients who acquired one or more infections during leukopenia was substantially reduced ($P<0.01$). This is evident for microbiologically as well as clinically documented infections (Table 2), especially in patients with less than 500 granulocytes (Table 3).

The reduction of the number of infections by selective decontamination of the digestive tract was especially remarkable in the respiratory and urinary tract (Table 6).

Table 6. Site and number of infections in the SDD treated and control group patients.

Site	Microbiologically and clinically documented infections	
	SDD	Control
Urinary tract	0	8
Respiratory tract	3	15
Pharynx	0	1
Blood	3	8
Skin/soft tissue	3	3
Anorectal	0	1
Others	0	2

Infection prevention seems to be possible both upon first,admission as well as during subsequent hospitalizations. There were 22 first hospitalizations in leukemic SDD patients, three patients developed five infections (one Gram-negative and one Gram-positive infection), while in the 20 leukemic patients without SDD 10 patients developed 22 infections (eight Gram-negative and two Gram-positive infections). During 13 subsequent hospitalizations in the SDD leukemic group two patients had two infections, while during 13 study periods in the control leukemic group three patients developed six infections (four Gram-negative and one Gram-positive infection).

The percentage of days (4%) with an axillary temperature above 38.5°C was significantly reduced in the group of SDD patients as compared to the percentage (15%) in the control group.

Death due to acquired infection. Nine of the 52 control patients but none of the 53 SDD patients died of infection ($P<0.01$).

Side effects. Nausea occurred frequently but in no case necessitated discontinuation of SDD. One patient treated with nalidixic acid developed phototoxicity and three patients developed skin rashes. No patients developed skin rashes during treatment with co-trimoxazole alone; in one such patient with a skin rash, more than one drug could have been responsible.

DISCUSSION

In this report an effective method for infection prevention without the use of a protected environment in granulocytopenic patients is presented. The method is based on the concept of the colonization resistance (21, 22). The colonization resistance is a natural barrier maintained by certain species of the anaerobic flora which prevents colonization and overgrowth by acquired contaminating potentially pathogenic microorganisms. Thus, for selective suppression or elimination of aerobic Gram-negative bacteria as well as of yeasts and fungi, we used only those antimicrobial drugs (nalidixic acid, co-trimoxazole, polymyxin E and amphotericin-B) which leave the anaerobic flora unaffected, even when applied in doses which are sufficiently high to eliminate the sensitive aerobic species. We have called this method selective decontamination of the digestive tract (SDD).

"Total bowel sterilization" was considered to be hazardous without protective environment, particularly because only CR-decreasing antibiotics such as neomycin and cephaloridin were available for this purpose. Following successful "total bowel sterilization" with these antibiotics, a substantial number of patients may acquire resistant strain of Gram-negative rods, and yeasts or fungi (44). Even when these strains are still sensitive to the antibiotics used for total decontamination, they often persist in the bowel (45). When the contaminant is resistant it may cause a rapid and massive overgrowth in the g.i. tract, which in turn may result in invasion and possibly a life-threatening infection (46). As a consequence of the increased risk of acquisition of resistant strains - decreased threshold dose for colonization - total bowel sterilization must be combined with a protected environment in order to reduce the chance of an unfortunate contamination.

During selective decontamination, i.e., oral administration of drugs for which the aerobic flora was sensitive, overgrowth with orally acquired sensitive or resistant contaminants from the hospital food or other environmental sources did not occur in our study. As our patients (the control group as well as the SDD group) were treated on an open ward and received the normal non-sterile hospital food, contamination by new exogenous Gram-negative rods will have occurred daily. This was confirmed by our routine three weekly culturing which showed continuously appearing and disappearing Gram-negative microorganisms in the throats of the control patients as well as a changing potentially pathogenic flora in the stool of these patients during their hospital stay.

In our SDD treated patients the contamination by exogenous Gram-negative microorganisms has only occasionally resulted in positive cultures of oropharynx swabs and feces. Furthermore, positive cultures regarding Gram-negative bacteria of throat swabs and feces in no case persisted for a week or longer (colonization), not even in those cases in which the contaminating microorganisms were resistant to the antimicrobial drugs used. This observation together with a constant concentration of the enterococci in the stool strongly suggests that the colonization resistance in our SDD patients remained intact, preventing persistence after the acquisition of new microorganisms. These results also indicate that an intact C.R. is equally effective for both resistant and sensitive contaminating microorganisms. In a separate paper comprehensive data are published on the bacteriological data (43).

Two of the antimicrobial drugs used in our study, co-trimoxazole and nalidixic acid, are well absorbed and may, in addition to their SDD effect, have had an additional systemic effect. The reduced number of positive cultures in patients with clinical infections could be due to the systemic effect of co-trimoxazole and nalidixic acid. However, this potential bias on the evaluation of SDD *per se* does not change our results. Furthermore, it is noteworthy that the number of clinical (not bacteriologically documented) infections was also reduced by SDD.

A possible disadvantage of the use of these prophylactic antimicrobial drugs could be the development of resistance among Gram-negative bacteria. However, in these patients this was not found to occur significantly (43), although incidental acquisition of resistant strains did occur. In no case did these invariably short "colonizations" lead to an infection or to an increase of episodes with "fever of unknown origin".

An obvious side effect of nalidixic acid and to some extent of amphotericin-B was the occurrence of nausea. This was of particular importance in the patients who were given chemotherapy with cytostatic drugs, which in themselves usually cause nausea and vomiting.

The anti-fungal part of the SDD medication consisted of oral amphotericin-B suspension. This was given in order to prevent yeast colonization in the gastrointestinal tract, which might lead to yeast infections. The crux of yeast infections during infection prophylaxis with co-trimoxazole has been reported by Hughes (47). However, Hughes used considerably higher doses of 12 tablets per day, aimed at prevention of *Pneumocystis carinii* infection. High doses of co-trimoxazole may affect the CR and therefore enhance Candida colonization. As judged from the occurrence of yeast infections (mainly Candida lesions in the oropharynx), a reduction of these lesions was seen in the SDD positive patients (P=0.051). This means that as a result of both the maintenance of a good colonization resistance as well as of oral amphotericin-B treatment, Candida infections did not constitute a major problem.

The beneficial effects of selective decontamination of the digestive tract with nalidixic acid or co-trimoxazole or polymyxin were recently confirmed by others. In the Pneumocystis prophylaxis studies of co-trimoxazole by Hughes e.a. (47) it was found that in children with acute lymphocytic leukemia, the use of co-trimoxazole prevented Gram-negative bacterial infections as well as Pneumocystis. Recently, the results of prophylactic co-trimoxazole in hospitalized granulocytopenic patients were published by Gurwith e.a. (48). However, only Guiot and Van Furth (49) used antimicrobial agents based on the concepts of selective decontamination in infection prevention.

Since we have shown that SDD can be effective in infection prevention and can decrease the number of deaths related to infection, we feel that this method may be preferable to total decontamination plus protective isolation. This view is primarily based on differences in the patient's comfort in treatment, convenience for the staff and in the cost. In our opinion SDD appears to be a very promising and possibly more widely applicable method for infection prevention in granulocytopenic patients.

ACKNOWLEDGEMENT

We thank B. Houwen, M.D. for his critical advice. We gratefully acknowledge the technical assistance of Miss Tine Polling and Mrs. W.J. A. Bruins-van der Weij.

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SUMMARY

Clinical signs and symptoms of deficiency of peripheral blood cells - red cells, granulocytes, monocytes and platelets - as a result of an insufficient production of these cells in the bone marrow is characteristic of the syndrome of bone marrow failure. Aplastic anaemia, characterized by a peripheral pancytopenia and bone marrow hypocellularity, can be distinguished from the syndrome of bone marrow insufficiency. However, aplastic anaemia also seems to encompasse a heterogeneous group of diseases with multiple aetiologies and with a variety of therapeutic approaches. In the introduction some of these aspects of aplastic anaemia are outlined.

In chapter I the occurrence of pancytopenia in family members of 8 patients with aplastic anaemia is described. In some cases, there seems to be a relation with the use of drugs or chemicals known to be associated with aplastic anaemia. In the other patients no known aetiological agents have been found. Presumably these families have a genetically determined susceptibility of the bone marrow for various known and unknown toxic agents. It is concluded that haematological investigations in family members of patients with aplastic anaemia are advisable, especially if bone marrow transplantation is considered.

In chapter II two patients with acquired aplastic anaemia are described who developed acute leukaemia after a number of years. The relationship which can be present between aplastic anaemia and acute leukaemia is discussed.

In chapter III a number of prognostic systems is evaluated in 43 patients with aplastic anaemia. The prediction of the prognosis of these patients is important in order to select from the currently available therapeutic approaches. The most reliable criteria for the recognition of patients dying within six months of diagnosis seems to be the criteria for severe aplastic anaemia described by Camitta et al. A further distinction in the group of patients who will survive for more than six months seems to be possible: decreasing granulocytes and platelets within three months of diagnosis are correlated with a survival less than 5 years.

Chapter IV describes the results of a controlled randomized clinical trial on the efficacy of selective decontamination of the digestive tract (SDD) as a method of infection prevention in granulocytopenic patients. This method consists of oral administration of antimicrobial agents (nalidixic acid, co-trimoxazole, polymyxin E and amphotericine B). These agents eliminate potentially pathogenic microorganisms in the digestive tract, while the

anaerobic part of the gutflora remains unaffected. The anaerobic micro-organisms are important for maintaining the integrity of the colonization resistance, the natural barrier against colonization of potentially pathogenic microorganisms after oral contamination. Suppression or elimination of the anaerobic flora by the application of non-selective antimicrobial agents can lead to colonization with resistant microorganisms, followed by severe infections. In the above mentioned study, patients treated with SDD acquired significantly less infections compared with untreated controls. No patients died as a result of infections. Despite treatment in open ward rooms in which the patients used normal, non-sterilized hospital food no bacterial overgrowth was found. This indicates that the colonization resistance remained intact in the SDD treated patients. It is concluded that selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients is worthwhile and may be preferable to total gut sterilization which necessitates isolation.

SAMENVATTING

Klinische verschijnselen van tekort aan bloedcellen - erytrocyten, granulocyten, monocyten en thrombocyten - als gevolg van onvoldoende productie van deze celsoorten in het beenmerg zijn kenmerkend voor het syndroom van de beenmerg insufficientie. Binnen dit syndroom kan het ziektebeeld van de aplastische anemie, bestaande uit pancytopenie en een hypoplastisch of aplastisch beenmerg, worden onderscheiden. Maar ook aplastische anemie lijkt een verzamelnaam te zijn voor een groep verschillende ziekten die het klinische beeld gemeen hebben, maar die op een verschillende manier zijn ontstaan en die misschien ook op verschillende wijze moeten worden behandeld. In de introduktie worden een aantal van deze aspecten van aplastische anemie beschreven.

In hoofdstuk I wordt het voorkomen van verworven pancytopenie bij familieleden van 8 patienten met aplastische anemie beschreven. In een aantal gevallen lijkt er een relatie te bestaan tussen de pancytopenie en het gebruik van geneesmiddelen die het beenmerg kunnen beschadigen, maar in een aantal andere kon geen bekend etiologisch agens worden gevonden. Er bestaat mogelijk in deze families een genetisch bepaalde gevoeligheid van het beenmerg voor al of niet bekende beenmerg toxische agentia. Geconcludeerd wordt dat het raadzaam is hematologisch onderzoek te verrichten bij familieleden van patienten met aplastische anemie, vooral wanneer beenmergtransplantatie wordt overwogen.

In hoofdstuk II worden 2 patienten met verworven aplastische anemie beschreven, die na verloop van enkele jaren een acute leukemie ontwikkelden. Ingegaan wordt op de mogelijke relatie die tussen deze ziektebeelden zou kunnen bestaan.

In hoofdstuk III worden een aantal in de literatuur beschreven prognostische criteria geëvalueerd bij 43 patienten met aplastische anemie. Het voorspellen van een prognose is van belang om zo een verantwoorde keuze te maken uit de thans beschikbare therapeutische mogelijkheden. De meest betrouwbare methode waarmee patienten met een levensduur minder dan zes maanden uit de gehele groep patienten met aplastische anemie kunnen worden geselecteerd, lijkt de diagnose „ernstige aplastische anemie" van Camitta e.a. te zijn. Deze auteurs gebruiken voor deze diagnose een granulocytenaantal kleiner dan $0{,}5 \times 10^9/l$ en een thrombocytenaantal kleiner dan $20 \times 10^9/l$. Op grond van onze eigen gegevens lijkt een verdere splitsing van de groep patienten met een levensduur langer dan zes maanden mogelijk. Wanneer het aantal granulocyten en het aantal thrombocyten drie maanden

nadat de diagnose aplastische anemie is gesteld, gedaald zijn ten opzichte van de uitgangswaarden is de levensverwachting van deze patienten korter dan vijf jaar.

In hoofdstuk IV worden de resultaten beschreven van een prospectief gerandomiseerd onderzoek naar de effectiviteit van selectieve darmdecontaminatie als methode om infecties te voorkomen bij patienten met granulocytopenie. Deze methode bestaat uit het oraal toedienen van antimicrobiële middelen (nalidixine zuur, cotrimoxazole, polymyxin E en amphotericine B), die de potentieel pathogene microörganismen in de tractus digestivus selectief elimineren, terwijl er geen nadelige invloed wordt uitgeoefend op de anaerobe microörganismen. Deze anaerobe microörganismen zijn van belang voor de kolonisatie resistentie van het maagdarmkanaal, een natuurlijke barriëre die bewerkstelligt dat orale besmettingen met pathogene microörganismen geen kans krijgen „aan te slaan". Wanneer echter door niet-selectieve antimicrobiële middelen ook de anaeroben worden gedood, is de kans op „overgroei" met (resistente) microörganismen groot, met als gevolg superinfecties. In het bovengenoemde onderzoek werd in de met selectieve darmdecontaminatie behandelde patienten een significante vermindering van het aantal infecties verkregen ten opzichte van de onbehandelde patienten. Bovendien stierven in deze groep geen patienten ten gevolge van een infectie, terwijl in de controle groep negen patienten zijn overleden als gevolg van een infectie. Ondanks verpleging op zaal werd door het intact laten van de kolonisatie resistentie van het maagdarmkanaal geen bacteriële overgroei of superinfecties in de behandelde patientengroep gezien. Geconcludeerd wordt dat de selectieve darmdecontaminatie als methode om infecties bij granulocytopenische patienten te voorkomen een grote aanwinst is, en dat deze methode de voorkeur verdient boven totale darmdecontaminatie die in een isolator moet plaatsvinden.